# Reagents for Pharma Industry

Chapter 3

PanReac AppliChem

ITW Reagents

# Summary

## Other Chapters of Reagents for Pharma Industry

**Chapter 1**
Physical and physicochemical methods (Ph. Eur. 2.2.) – Degree of coloration of liquids. Clarity and degree of opalescence of liquids. Potentiometric determination of pH. Approximate pH of solutions. Conductivity
Pharmaceutical technical procedures (Ph. Eur. 2.9.) – Dissolution test

**Chapter 2**
Physical and physicochemical methods (Ph. Eur. 2.2.) - Spectroscopy

**Chapter 4**
Physical and physicochemical methods (Ph. Eur. 2.2.) - Chromatography

**Chapter 5**
Physical and physicochemical methods (Ph. Eur. 2.2.) - Biochemistry

**Chapter 6**
Identification (Ph. Eur. 2.3.)
Limit tests (Ph. Eur. 2.4.)
Assays (Ph. Eur. 2.5.)

**Chapter 7**
Waste water analysis

**Chapter 8**
Synthesis

# About Us

## *The Origin*

ITW Illinois Tool Works Inc. (NYSE: ITW) is a global industry company that delivers specialized expertise, innovative thinking and value-added products to meet critical customer needs in a variety of industries.

ITW, with approximately 14 billion dollars in global revenues, operates 7 major segments with businesses in 58 countries that employ approximately 50,000 employees. The company has a broad portfolio of more than 17,000 global patents and patent applications.

## *The ITW Reagents Division*

In 2010, the ITW Reagents division was born integrated by the companies Panreac Química SLU (Spain) and Nova Chimica Srl (Italy), and later on by AppliChem GmbH (Germany). The division offers the highest quality and innovative products for analysis, research and production applications.

ITW Reagents markets its products worldwide through an extensive distribution network to more than 80 countries under the PanReac AppliChem brand. It has two production plants in Darmstadt (Germany) and Barcelona (Spain).

1912 ITW → 1941 Panreac Química → 1980 Nova Chimica → 1992 AppliChem

## *We are Everywhere*

We can say that almost all products subject to human manipulation have undergone chemical analysis that guarantees their physical and chemical properties. Food, agrifood, medicines, cosmetics... and so many other products are subjected to chemical analysis. Our reagents can be found in any quality control and research laboratory.

**Our range of Laboratory Chemicals include:**

Analytical reagents
Reagents for volumetric analysis
Reagents and solvents for general applications
Reagents and solvents for HPLC
Reagents and solvents for GC
Reagents for metallic traces analysis
Analytical standards
Reagents and solvents for specific applications
Products for clinical diagnosis
Products for microbiology

**Our range of Laboratory Biochemicals cover:**

Cell Biology / Cell Culture
Protein Biochemistry and Electrophoresis
Nucleic Acid Biochemistry
General Biochemicals and Biological Buffers
Special Biochemicals

## *Service & Benefits*

**Exceptional know-how** and a wide range of chemicals and biochemicals for a great diversity of applications.

**European production** committed to corporate social responsibility (CSR).

**Efficient global distribution network** to export our products worldwide to more than 80 countries.

**Qualified management team** fully committed to our business project.

## *Excellence*

Our products are strictly controlled in our laboratories and meet the highest quality requirements. A multi-site Integrated Management System for Quality, Environment and Safety is implemented in all activities and processes.

ISO 9001:2015 | ISO 14001:2015 | OHSAS 18001:2007

# Reagents for Pharma Industry
Chapter 3

## Introduction

The **Pharmaceutical Industry** discovers, develops, produces, and markets drugs or **pharmaceutical drugs** for use as medications.

Pharmaceutical companies may deal in **generic** or **brand medications** and medical devices.

They are subject to a variety of **laws** and **regulations** that govern the patenting, testing, safety, efficacy and marketing of drugs.

The pharmaceutical industry is largely driven by **scientific discovery** and **development**, in conjunction with **toxicological** and **clinical experience**.

Major differences exist between **large organizations** which engage in a broad range of drug discovery and development, manufacturing and quality control, marketing and sales and **smaller organizations** which focus on a specific aspect.

Most multinational pharmaceutical companies are involved in all these activities; however, they may specialize in one aspect based upon local market factors. Academic, public and private organizations perform scientific **research to discover and develop new drugs**. The biotechnology industry is becoming a major contributor to innovative pharmaceutical research. Often, collaborative agreements between research organizations and large pharmaceutical companies are formed to explore the potential of new drug substances.

**Active drug substances** (APIs, Active Principle Ingredient) and **inert materials** (Excipients) are combined **during pharmaceutical manufacturing** to produce dosage forms of medicinal products (e.g. tablets, capsules, liquids, powders, creams and ointments). Drugs may be categorized by their manufacturing process and therapeutic benefits.

The different pharmaceutical manufacturing processes each have their own **environmental issues** and the wastes must be treated and controlled. **For example:**

- During **fermentation process**, the spent fermentation broth contains sugars, starches, proteins, nitrogen, phosphates and other nutrients with high biochemical oxygen demand (BOD), chemical oxygen demand (COD) and total suspended solids (TSS) with pH values ranging from 4 to 8.
- Also, wastes from **chemical synthesis** are complex due to the variety of hazardous materials, reactions and unit operations. These waste waters are high in BOD, COD and TSS, with varying acidity or alkalinity and pH values ranging from 1 to 11.

**The analysis laboratories** play a fundamental role in the pharmaceutical industries. **They are key pieces in:**

- Discovery and improvement of a **drug.**
- Development and optimization of **manufacturing processes.**
- **Quality control** of raw materials, intermediates and finished products.
- Quality control of **wastes.**

!

Depending on the type of analysis in which they are involved, **different types of laboratories** can be distinguished within the same pharmaceutical company. Besides, the **type of analysis** and the techniques used may be different (as shown on the next page).

In any case, the methods of analysis must be strictly validated and follow the requirements set by the **Pharmacopoeias** (Ph. Eur., USP, etc.) both in the analysis protocols and in the quality of the reagents to be used.

Our **portfolio** includes a wide range of products such as solvents, acids, bases and salts indicated for general analytical applications that **fulfil the requirements indicated in the Pharmacopoeias** (Ph. Eur. or USP) for the reagents to be used for analytical purposes.

# Reagents for Pharma Industry

Chapter 3

## Types of Laboratories versus Methods of Analysis

| Facility | | R&D Centre | | Manufacturing Plant Quality Control | | | Wastewater Plant |
|---|---|---|---|---|---|---|---|
| Laboratory | | New molecules / Improvements of existing products | Analytical development | Raw Material (excipients & APIs) | In-process (intermediate product) | Final product | Water quality control |
| Methods of analysis | Chapter | | | | | | |
| Amino acid analysis | 5 | | | ● | ● | ● | |
| Ammonium | 6/7 | | | | | | ● |
| Approximate pH of solutions | 1 | | ● | ● | ● | ● | ● |
| Assay: Protein (Kjeldahl) | 6 | | ● | ● | | | |
| Assay: Titration | 6 | | ● | ● | | | |
| Assay: Water (KF) | 6 | | ● | ● | ● | ● | |
| Atomic Absorption spectroscopy | 2 | | ● | ● | | | |
| Biological assays | 3 | | ● | ● | | | |
| Biological tests | 3 | | | ● | | ● | |
| Clarity and opalescence of liquids | 1 | | ● | ● | | ● | |
| Chlorinated compounds | 7 | | | | | | ● |
| Conductivity | 1 | | ● | ● | | | |
| Degree of Coloration of Liquids | 1 | | ● | ● | | | |
| Detergents (Surfactants) | 7 | | | | | | ● |
| Dissolution Test | 1 | | | | | ● | |
| Electrophoresis | 5 | ● | ● | ● | ● | ● | |
| Gas Chromatography | 4 | ● | ● | ● | | ● | |
| ICP | 2 | | ● | ● | | | |
| Identification | 6 | | ● | ● | | ● | |
| IR | 2 | ● | ● | ● | | ● | |
| Limit tests | 6 | | ● | ● | | | |
| Liquid Chromatography | 4 | ● | ● | ● | | ● | |
| Molecular mass distribution in dextrans | 5 | | | ● | ● | | |
| Organic compounds (COD, DBO5, TOC) | 7 | | | | | | ● |
| Peptide identification by NMR spectrometry | 5 | ● | ● | ● | ● | ● | |
| Peptide mapping | 5 | ● | | ● | ● | ● | |
| Phosphates | 6/7 | | | | | | ● |
| Potentiometric determination of pH | 1 | | ● | ● | ● | ● | ● |
| Residual catalyzers (Metals, Cyanides) | 7 | | | | | | ● |
| Suspended matter | 7 | | | | | | ● |
| Thin Layer Chromatography | 4 | ● | ● | ● | | | |
| UV | 2 | ● | ● | ● | | | |
| Synthesis* | 8 | ● | | | | | |

*not a method of analysis but reagents and solvents involved in synthesis procedures.

In the following sections we will describe the most common methods of analysis indicated in the pharmacopoeias and offer the most appropriate reagents for each method.

The European Pharmacopoeia (Ph. Eur.) was conceived to have a secure production and to reach a better comparability. Biology plays a major role as medicines are developed for human and veterinary use.

The support that Microbiology provides to the pharmaceutical and cosmetics industry is very diverse. It participates in the development of vaccines and biologicals, as well as in the different microbiological production and analysis processes to guarantee the quality of pharmaceutical products.

So the tests in Ph. Eur. chapter 2.6 and 2.7 all refer to biological organisms or detection of biomolecules. We will cover this chapter to the extent that we can support you with material.

# Sterility

This test applies to substances, preparations or articles which, according to the Pharmacopoeia, are required to be sterile. However, a satisfactory result only indicates that no contaminating microorganisms were found in the sample examined under the test conditions.

The sterility test has to be performed under aseptic conditions.

Precautions taken to avoid contamination should be such that they do not affect any microorganisms to be revealed in the test. Working conditions should be monitored regularly.

## Culture media and incubation temperatures

The following culture media have been found to be suitable for the sterility test. Fluid thioglycollate medium is primarily intended for the culture of anaerobic bacteria; however, it can also detect aerobic bacteria. Soya bean-casein digest medium (TSB) is suitable for the culture of fungi and aerobic bacteria.

| Product name | Composition (g/l) | Code | Package |
|---|---|---|---|
| **Thioglycollate Liquid Medium (Ph. Eur., USP, ISO 7937) (Dehydrated Culture Media) for microbiology** | Sodium Thioglycollate ..... 0.5<br>L-Cystine ..... 0.5<br>Yeast Extract ..... 5.0<br>D(+)-Glucose (anhydrous) ..... 5.0<br>Enzymatic Digest of Casein ..... 15.0<br>Resazurin ..... 0.001<br>Sodium Chloride ..... 2.5<br>Agar ..... 0.75<br>pH 7.1 ± 0.2 | 413912.1210 | 500 g |
| **Tryptone Soy Broth (TSB) (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Papaic Digest of Soya ..... 3.0<br>D(+)-Glucose ..... 2.5<br>Digest Pancreatic of Casein ..... 17.0<br>di-Potassium Hydrogen Phosphate ..... 2.5<br>Sodium Chloride ..... 5.0<br>pH 7.3 ± 0.2 | 413820.1210 | 500 g |
| | | 413820.0914 | 5 kg |

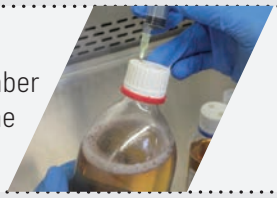
*Fluid thioglycollate medium has to be incubated at 30 - 35 °C*
*Soya-bean casein digest medium (TSB) has to be incubated at 20 - 25 °C*

The media used have to comply with the following tests carried out prior to or in parallel with the test on the product to be examined.

**Sterility:** no microorganisms growth within 14 days.

**Growth promotion test of aerobes, anaerobes and fungi:** the media are suitable if clearly visible growth of microorganisms occurs.

Inoculate portions of fluid thioglycollate with small number (not more than 100 CFU) of the following microorganisms,



**Aerobic bacteria**
*Staphylococcus aureus*
*Pseudomonas aeruginosa*

**Anaerobic bacterium**
*Clostridium sporogenes*

Inoculate portions of soya-bean casein digest medium with a small number (not more than 100 CFU) of the following microorganisms,

**Aerobic bacteria**
*Bacillus subtilis*

**Fungi**
*Candida albicans*
*Aspergillus brasiliensis*

Incubate for not more than 3 days in the case of bacteria and not more than 5 days in case of fungi.

## Method suitability test

This test is performed:

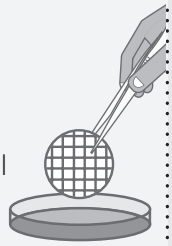
**a.** When the test for sterility has to be carried out on a new product;

**b.** Whenever there is a change in the experimental conditions of the test.

The method suitability test may be performed simultaneously with the test for sterility of the product to be examined. Sample analysis can be carried out exactly the same except for the following modifications:

**Membrane filtration**

After tranferring the contents of the containers to be tested to the membrane add an inoculum of a small number of viable microorganisms (no more than 100 CFU) to the final portion of sterile diluent used to rinse the filter.



**Direct inoculation**

After tranferring the contents of the containers to be tested to the culture medium add an inoculum of a small number of viable microorganisms (no more than 100 CFU) to the medium.

In both cases the same microorganisms are used (aerobic bacteria, anaerobic bacteria and fungi).
A positive promotion test has to be carried out with an incubation time of no more than 5 days.

**Results**

| | | | | |
|---|---|---|---|---|
| **Clearly visible growth** | → | Comparable to the control | → | No antimicrobial activity: Sterility test can be carried out without further modification |
| **Not clearly visible growth** | → | Product posseses antimicrobial activity | → | Modify conditions in order to eliminate antimicrobial activity and repeat the method suitability test |

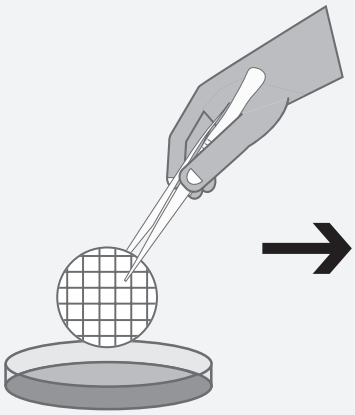
## Test for sterility of the product to be examined

Sterility test of the product may be carried out using the technique of **membrane filtration** or **direct inoculation**. Appropriate negative controls are included.

### Membrane filtration

Whenever the nature of the sample permits it (aqueous, alcoholic, oily preparations and preparations with solvents without antimicrobial effects).

Use membrane filters no greater than 0.45 µm and approximately 50 mm diameter, suitable for the sample to be filtered, using a sterilised filtration apparatus.



- Aqueous solutions
- Soluble solids
- Oils and oily solutions
- Ointments and creams

### Direct Inoculation of the culture medium

1. Transfer the quantity of preparation described in the pharmacopoeia directly into the culture medium so that the volumen of the product is not more than 10 % of the volumen of the medium.
2. Incubate the inoculated media not less than 14 days. Observe the cultures several times during the incubation period.

- Oily liquids
- Ointments and creams
- Catguts an other surgical sutures for veterinary use

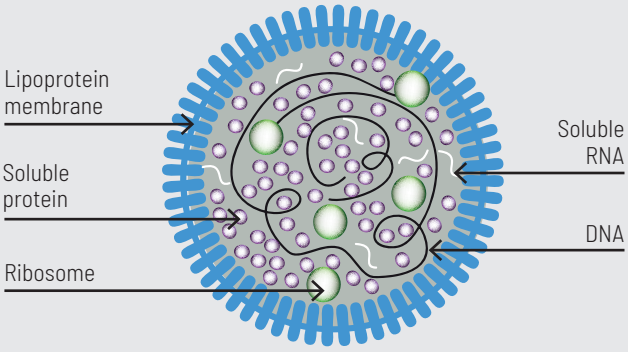
## Observation and Interpretation of results

Examine the media for macroscopic evidence of microbial growth → Turbidity → Transfer not less than 1 ml incubation portion to fresh medium and incubate not less than 4 days

**Results**

**No evidence of microbial growth** ⟶ Product complies with sterility test

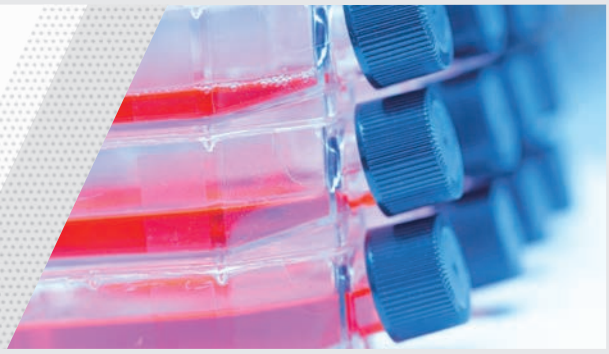
# Mycoplasma control




**Mycoplasma control** is especially important in the field of medicine. Therefore, testing against mycoplasma is an essential requirement for pharmaceuticals.

There are two standard methods of detection. One is by incubation of samples under special growth conditions for mycoplasma. Second is the **detection via nucleic acid amplification techniques.**

Surveys of cultures from labs all over the world reveal a strong prevalence of contamination by mycoplasma and other mollicutes. Depending on the method of detection 10-40% of continuous cell lines have been tested positively. The species most frequently found are *Mycoplasma orale, M. fermentans* (human), *M. arginini, Acholeplasma laidlawii* (bovine), and *M. hominis* (swine).



### Sources of contamination

There are various possible sources for contamination by mycoplasmas. During recent years, a rising awareness of the problem may have changed the contribution of the individual sources. Culture reagents such as bovine serum have been a considerable source of contamination in the past. Today, most labs prefer mycoplasma-free tested sera. Laboratory personnel, who may introduce mycoplasmas into cultures, are now trained to avoid contamination during the handling of cultures. However, other sources are even more difficult to avoid. Any addition to the culture is relevant, such as virus suspensions, antibody solutions, or media ingredients. Mycoplasmas from original tissue isolates contribute to less than 1% to the reported cases. The most common source by far is cross-contamination from infected cultures. Labs exchange infected cultures and thereby inadvertently distribute mycoplasmas.

PanReac AppliChem provides the tools for detection and treatment of mycoplasmas for every cell culture laboratory. For the detection by microscopy we are offering the proven fluorescent dye DAPI (product code A1001, available in pack sizes from 10 mg to 10 g).

### Detection by PCR

In recent years the sensitive polymerase chain reaction (PCR) became a standard method for the detection of mycoplasma contamination in biological samples such as mammalian cell cultures. The PCR is established in almost all life science labs either as standard PCR or real time/quantitative PCR. For your preferred setup, we offer three different kits to choose from.

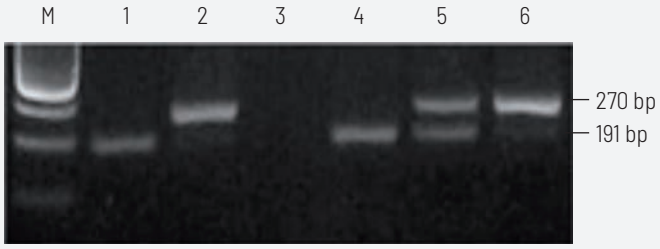
The rRNA gene sequences of prokaryotes including mycoplasmas are well conserved, whereas the lengths and sequences of the spacer region in the rRNA differ from species to species. The detection procedure utilizes the PCR for amplification of a conserved and mycoplasma-specific 16S rRNA gene region. This system does not allow the amplification of DNA originating from other sources, such as cultured cells or bacteria, which affect the detection result. Amplification of the gene sequence with PCR using this primer set enhances not only the sensitivity, but also the specificity of detection. Amplified products are detected by agarose gel electrophoresis or by real time/quantitative PCR (qPCR Mycoplasma Test Kit, product code A9019).

# Mycoplasma in cell culture - detection and elimination

## Mycoplasma detection kits using standard PCR

| Product name | Kit components | Taq DNA polymerase | Storage | Code | Package |
|---|---|---|---|---|---|
| **PCR Mycoplasma Test Kit** | • Reaction Mix (PCR primers, dNTPs, Taq DNA polymerase)<br>• Buffer solution<br>• Positive template control.<br>Ready-to-use master mix, liquid | Included | -20 °C | A3744,0020 | 20 tests |
| **PCR Mycoplasma Test Kit II**<br><br>This kit meets criteria of Ph. Eur. section 2.6.7. | • Reaction Mix (including PCR primers, dNTPs)<br>• Reaction Buffer Solution<br>• PCR grade water<br>• Positive template control<br>• Internal control DNA.<br>Single components, lyophilized | Not included* | 2 – 8 °C | A8994,0025 | 25 tests |
| | | | | A8994,0050 | 50 tests |
| | | | | A8994,0100 | 100 tests |
| **qPCR Mycoplasma Test Kit** | • Reaction Mix (including PCR primers, dNTPs)<br>• Reaction Buffer Solution<br>• PCR grade water<br>• Positive template control<br>• Internal control DNA.<br>Single components, lyophilized | Included | 2 – 8 °C | A9019,0025 | 25 tests |

** Use kit A8994 in combination with hot-start polymerase. We recommend SuperHot Taq DNA polymerase, code A5231.*



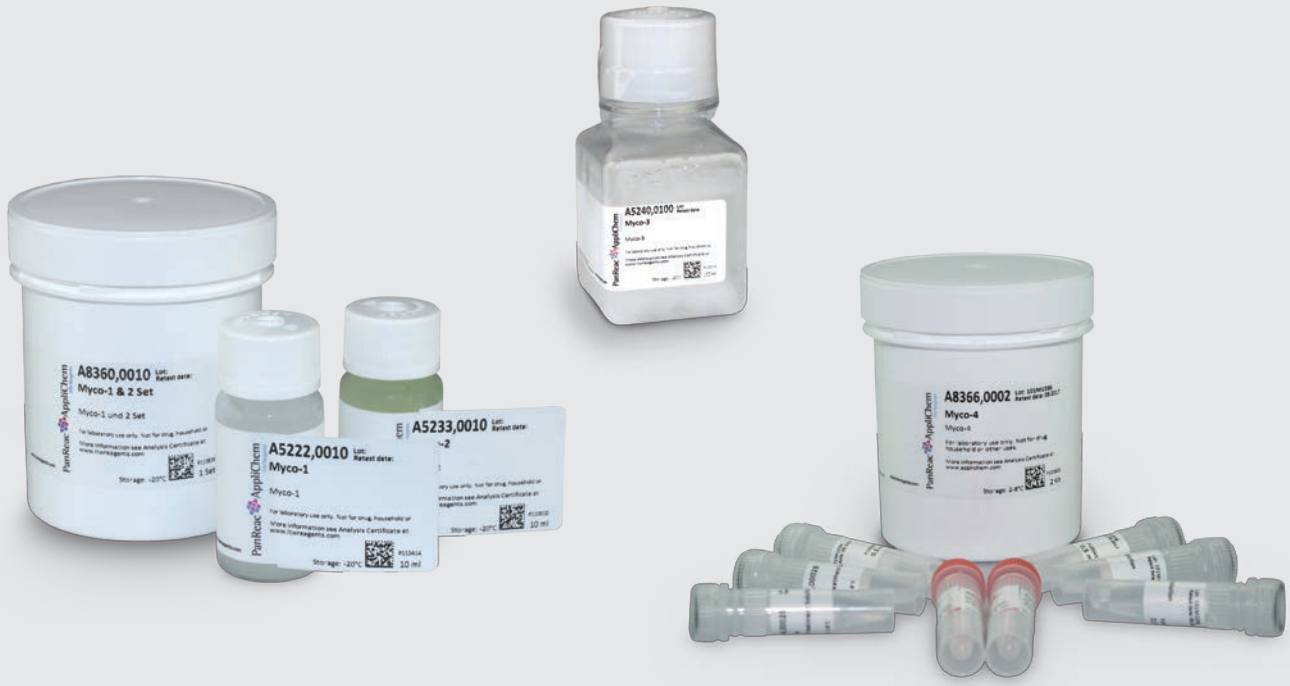

Possible PCR products of PCR Mycoplasma Test Kit II

1: negative control
2: positive control
3: inhibited sample
4: negative sample
5: contaminated positive sample
6: contaminated positive sample with high mycoplasma DNA concentration
M: DNA marker


## Treatment of Mycoplasma Infections in Cell Cultures

PanReac AppliChem offers well-proven treatments to achieve reliable elimination of mycoplasma infections from mammalian cell cultures. Precious cell cultures that are infected cannot always be simply discarded and replaced by new ones. For both, biological and economical reasons it is important to eliminate mycoplasma from cell cultures used in basic research, diagnostics, and biotechnological production.

| Product name | Application | Kit components | Storage | Code | Package |
|---|---|---|---|---|---|
| **Myco-1 & 2 Set** | For the treatment of all mammalian cell lines including embryonic stem cells (ES cells). Both agents are used in combination, one after another. | • Myco-1 (A5222), based on the antibiotic Tiamulin<br>• Myco-2 (A5233), based on the antibiotic Minocycline<br>Sterile 100X concentrated antibiotic solutions | -20 °C | A8360,0010 | 1 Set (2 x 10 ml) |
| **Myco-3** | Eliminates the most common mycoplasma contaminants including *M. orale, M. hyorhinis, M. fermentans, M. arginini, as well as A. laidlawii.* | • Myco-3 is based on the antibiotic Ciprofloxacin | -20 °C | A5240,0010 | 10 ml |
| | | | | A5240,0020 | 20 ml |
| | | | | A5240,0100 | 100 ml |
| **Myco-4** | Novel combination of antibiotic and biophysical agents. For maximum efficiency and a broad spectrum. Almost 100 % of permanent eradication of mycoplasma is achieved. | Each kit contains:<br>• 1 vial of Starter Treatment solution<br>• 3 vials of Main Treatment solution<br>One kit is needed for a treatment | 2 – 8 °C | A8366,0002 | 2 kits |



# Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests

The tests described in this section allow **quantitative enumeration of mesophilic bacteria**, total aerobic microbial count **(TAMC)** and **fungi**, total combined yeasts and molds count **(TYMC)**, that can grow under aerobic conditions.

The tests are primarily designed to determine whether a substance or preparation meets an established microbiological quality specification.

These methods are not applicable to products containing viable microorganisms as active ingredients.

### General procedures

The conditions of the analysis must be done avoiding external contamination. If the product to be examined contains antimicrobial activity, this has to be removed or neutralised. If inactivators are used for this purpose, the efectiveness of these has to be demonstrated.

### Enumeration methods

Membrane filtration or plate-count method can be used. Most Probable Number (MPN) can be used as the least accurate method but for certain products it may be the most appropriate method.

### Growth promotion test, suitability of the counting method and negative controls

The ability of the test to detect microorganisms in the presence of product to be tested must be established. For this purpose, a standarised stable suspensions of strains must be used (as indicated in pharmacopoeias) and a negative control must be done to verify testing conditions.

### Growth promotion of the media

Each batch of prepared and dehydrated media should be tested using standardized strains. For solid media, the growth obtained must not differ by more than a factor of 2 from the calculated value for a standardised inoculum.

A test of the suitability of the counting method in the presence of the product should also be performed.

# Procedures for determining microbial count: TAMC (Total Aerobic Microbial Count) and TYMC (Total Combined Yeasts and Molds Count)

## Description

Quantitative count of mesophilic bacteria and fungi that can develop under aerobic conditions.

## Media and reagents

To prepare stock solution:

- Buffered Solution of Sodium Chloride-Peptone pH 7.0
- Phosphate Buffer Solution pH 7.2
- Casein Soya Bean Digest Broth (Tryptone Soy Broth)

Media for counting bacteria:

- Casein Soya Bean Digest Agar (Tryptone Soy Agar)
- Casein Soya Bean Digest Broth (Tryptone Soy Broth)

Media for counting fungi and yeasts:

- Sabouraud Glucose Agar
- Dextrose Potato Agar (Potato Glucose Agar)
- Sabouraud Glucose Agar with antibiotics (for those cases where the TYMC count is expected to exceed due to bacterial growth)

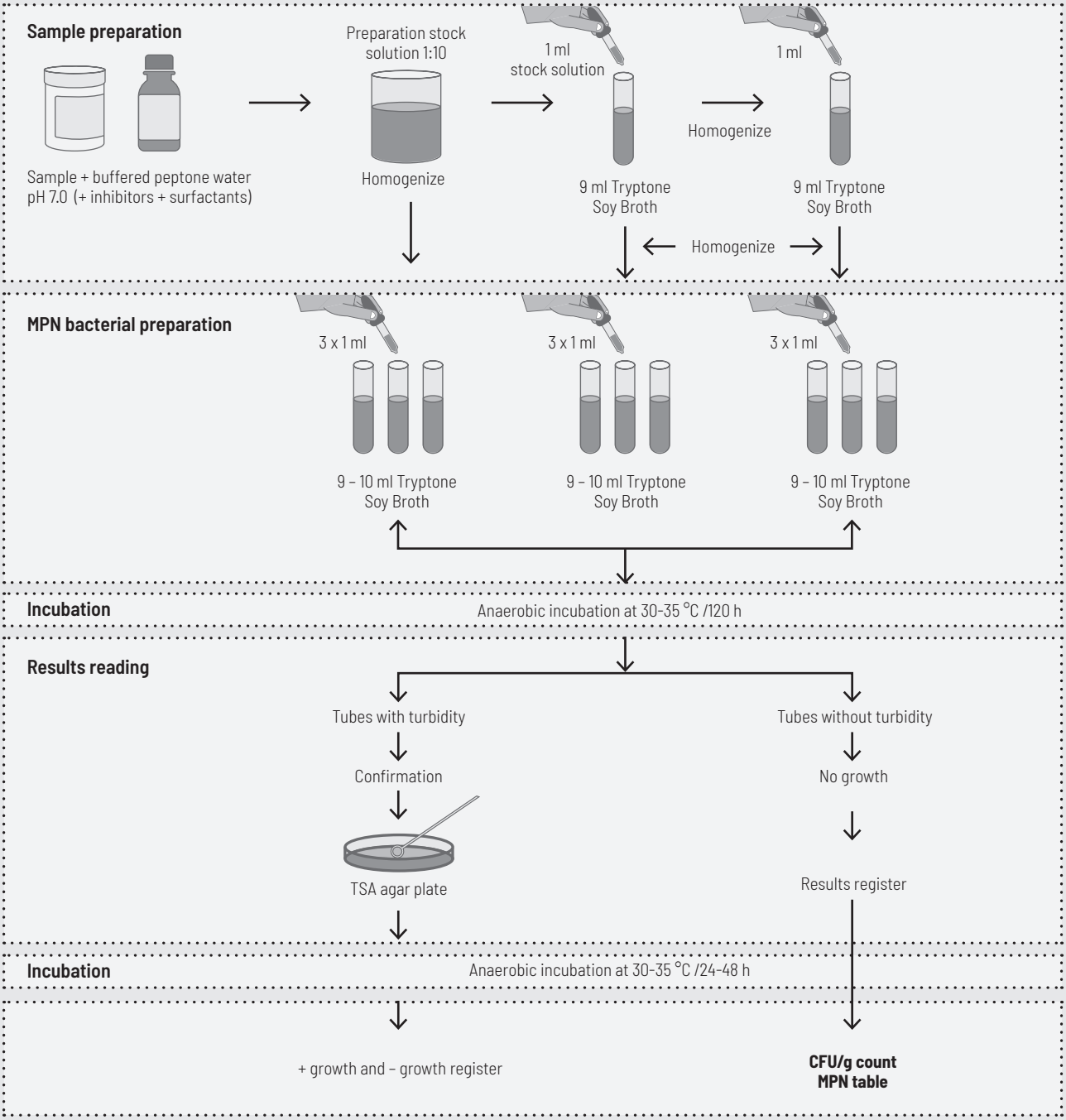
| Product name | Composition (g/l) | Code | Package |
|---|---|---|---|
| **Buffered Sodium Chloride-Peptone solution (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Digest Pancreatic of Casein ..... 1.00<br>Potassium di-Hydrogen Phosphate ..... 3.60<br>Sodium Chloride ..... 4.30<br>di-Sodium Hydrogen Phosphate 2-hydrate ..... 7.20<br>pH 7.0 ± 0.2 | 414944.1210 | 500 g |
| | | 414944.0914 | 5 kg |
| **PBS tablets pH 7.2** (for 1 L) | Phosphate ..... 10 mM<br>NaCl ..... 140 mM<br>KCl ..... 2.7 mM | A9202,0010 | 10 tablets |
| | | A9202,0100 | 100 tablets |
| **Potato Glucose Agar (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | D(+)-Glucose ..... 20.0<br>Potatoes Infusion (200 g) ..... 4.0<br>Agar ..... 15.0<br>pH 5.6 ± 0.2 | 413758.1210 | 500 g |
| **Tryptone Soy Agar (TSA) (Ph. Eur.)** | Papaic Digest of Soya ..... 5.0<br>Digest Pancreatic of Casein ..... 15.0<br>Sodium Chloride ..... 5.0<br>Agar ..... 15.0<br>pH 7.3 ± 0.2 | 413819.1210 | 500 g |
| | | 453819.0922 | 20 plates 90 mm |
| **Tryptone Soy Broth (TSB) (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Papaic Digest of Soya ..... 3.0<br>D(+)-Glucose ..... 2.5<br>Digest Pancreatic of Casein ..... 17.0<br>di-Potassium Hydrogen Phosphate ..... 2.5<br>Sodium Chloride ..... 5.0<br>pH 7.3 ± 0.2 | 413820.1210 | 500 g |
| | | 413820.0914 | 5 kg |
| **Sabouraud Glucose Agar (Ph. Eur.)** | D(+)-Glucose ..... 40.0<br>Mixture of Peptic Digest of Animal Tissue and Pancreatic Digest of Casein (1:1) ..... 10.0<br>Agar ..... 15.0<br>pH 5.6 ± 0.2 | 413802.1210 | 500 g |
| | | 453802.0922 | 20 plates 90 mm |
| **Sabouraud Glucose Agar+Chloramphenicol (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | D(+)-Glucose ..... 40.0<br>Chloramphenicol ..... 0.05<br>Mixture of Peptones ..... 10.0<br>Agar ..... 15.0<br>pH 5.6 ± 0.2 | 413842.1210 | 500 g |
| | | 433842.0922 | 30 contact plates |

## Method

There are 4 different procedures:

- Most Probable Number (MPN)
- Pour-plate method
- Surface-spread method
- Membrane filtration

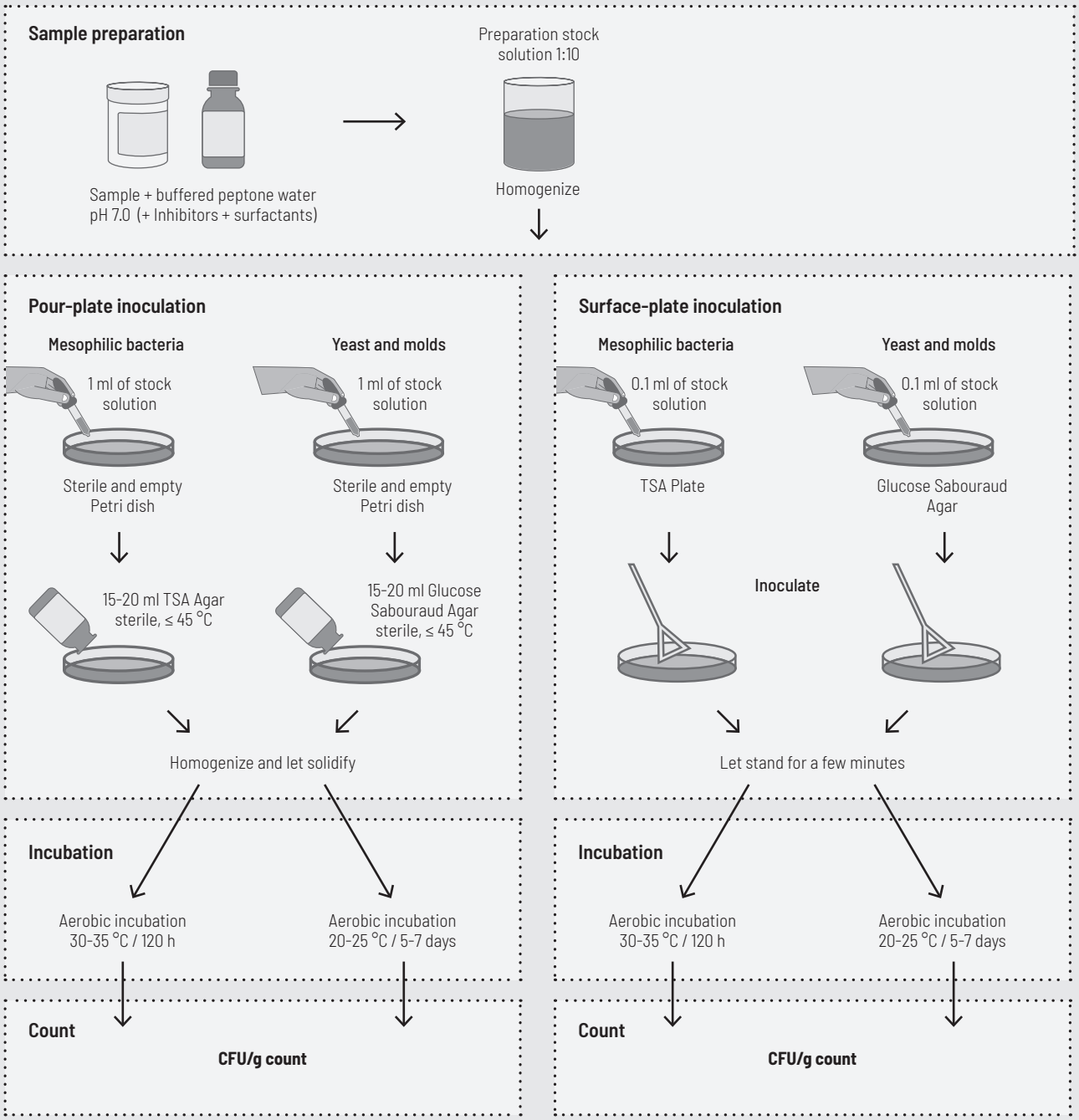
Most Probable Number (MPN)
3 sets of 3 tubes



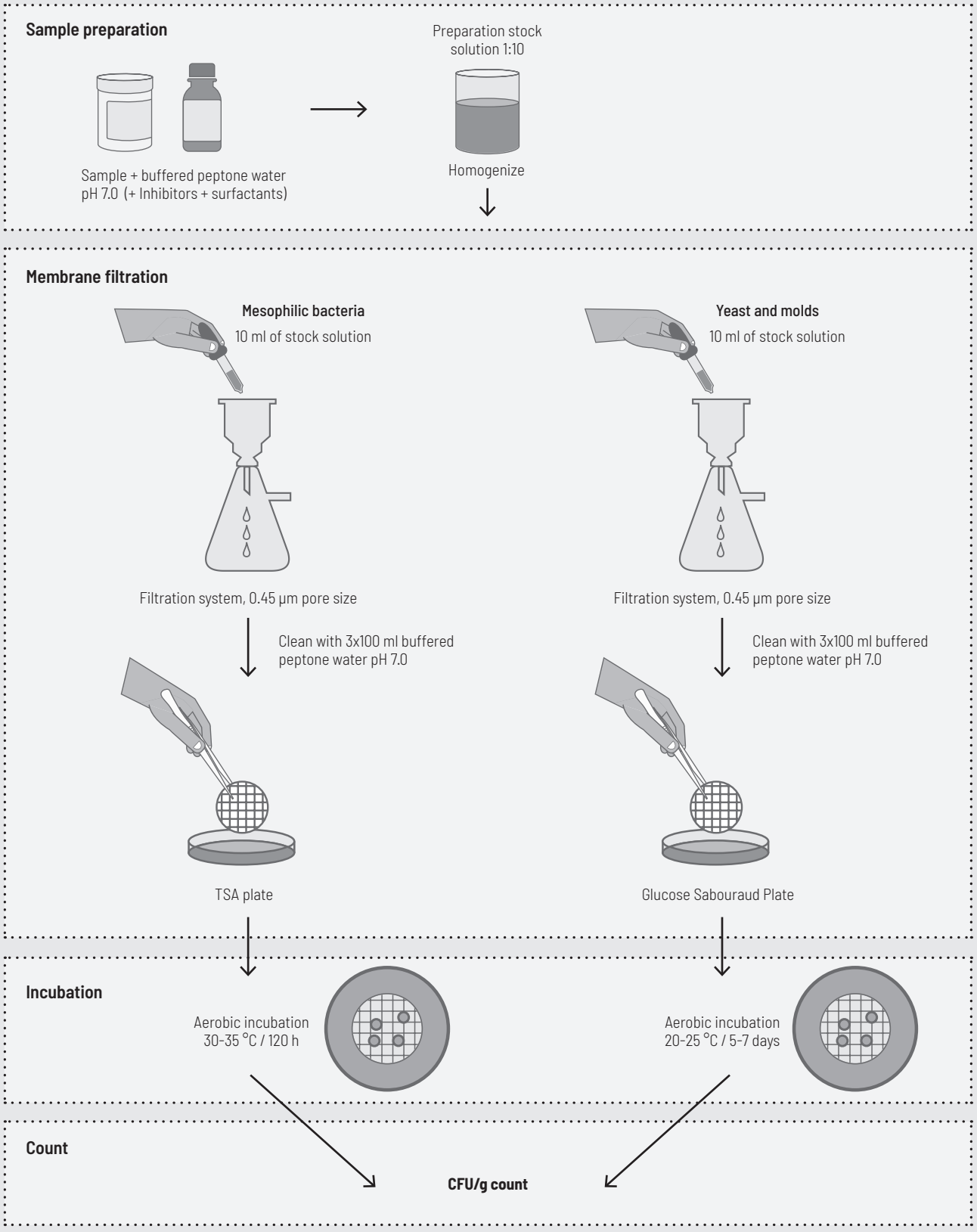
## Most-Probable-Number Values of Microorganims

| Observed combinations of numbers of tubes showing growth in each set | | | MPN per g or mL of product | 95 % confidence limits |
|---|---|---|---|---|
| Number of g or mL of product per tube | | | | |
| 0.1 | 0.01 | 0.001 | | |
| 0 | 0 | 0 | < 3 | 0 - 9.4 |
| 0 | 0 | 1 | 3 | 0.1 - 9.5 |
| 0 | 1 | 0 | 3 | 0.1 - 10 |
| 0 | 1 | 1 | 6.1 | 1.2 - 17 |
| 0 | 2 | 0 | 6.2 | 1.2 - 17 |
| 0 | 3 | 0 | 9.4 | 3.5 - 35 |
| 1 | 0 | 0 | 3.6 | 0.2 - 17 |
| 1 | 0 | 1 | 7.2 | 1.2 - 17 |
| 1 | 0 | 2 | 11 | 4 - 35 |
| 1 | 1 | 0 | 7.4 | 1.3 - 20 |
| 1 | 1 | 1 | 11 | 4 - 35 |
| 1 | 2 | 0 | 11 | 4 - 35 |
| 1 | 2 | 1 | 15 | 5 - 38 |
| 1 | 3 | 0 | 16 | 5 - 38 |
| 2 | 0 | 0 | 9.2 | 1.5 - 35 |
| 2 | 0 | 1 | 14 | 4 - 35 |
| 2 | 0 | 2 | 20 | 5 - 38 |
| 2 | 1 | 0 | 15 | 4 - 38 |
| 2 | 1 | 1 | 20 | 5 - 38 |
| 2 | 1 | 2 | 27 | 9 - 94 |
| 2 | 2 | 0 | 21 | 5 - 40 |
| 2 | 2 | 1 | 28 | 9 - 94 |
| 2 | 2 | 2 | 35 | 9 - 94 |
| 2 | 3 | 0 | 29 | 9 - 94 |
| 2 | 3 | 1 | 36 | 9 - 94 |
| 3 | 0 | 0 | 23 | 5 - 94 |
| 3 | 0 | 1 | 38 | 9 - 104 |
| 3 | 0 | 2 | 64 | 16 - 181 |
| 3 | 1 | 0 | 43 | 9 - 181 |
| 3 | 1 | 1 | 75 | 17 - 199 |
| 3 | 1 | 2 | 120 | 30 - 360 |
| 3 | 1 | 3 | 160 | 30 - 380 |
| 3 | 2 | 0 | 93 | 18 - 360 |
| 3 | 2 | 1 | 150 | 30 - 380 |
| 3 | 2 | 2 | 210 | 30 - 400 |
| 3 | 2 | 3 | 290 | 90 - 990 |
| 3 | 3 | 0 | 240 | 40 - 990 |
| 3 | 3 | 1 | 460 | 90 - 1980 |
| 3 | 3 | 2 | 1100 | 200 - 4000 |
| 3 | 3 | 3 | > 1100 | |

## Pour-plate and Surface-spread methods



## Membrane filtration technique



# Microbiological Examination of Non-Sterile Products: Tests for Specified Microorganisms

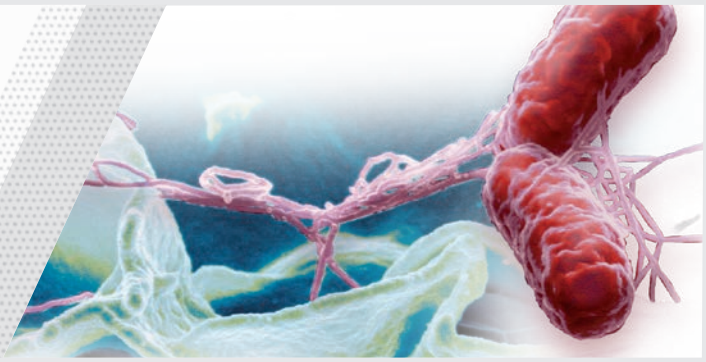
The tests described in this section determine the absence or **limited occurrence of certain microorganisms that may be detected under specific conditions.**

Membrane filtration or plate-count method can be used. Most probable number can be used as the least accurate method but for certain products it may be the most appropriate method.

Standardized strains must be used to evaluate ready-to-use and dehydrated culture media. Use Sodium Chloride-Peptone pH 7.0 (code 414944), or PBS pH 7.2 (code A9202) to make tests suspensions. The suspensions must be used within 2 h or within 24 h if stored at 2 - 8 °C.

The specified microorganisms to be tested are:

- *Bile-Tolerant Gram-Negative Bacteria*
- *Escherichia coli*
- *Salmonella sp.*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Clostridia*
- *Candida albicans*



Ready-to-use Contact Plates

Ready-to-use Media Plates (90 mm)


Dehydrated Culture Media (powder)

# Bile-Tolerant Gram-Negative Bacteria

## Description

Qualitative and semiquantitative study of microorganisms of the family of *Enterobacteriaceae* and of certain types of Gram-negative organisms *(Aeromonas, Pseudomonas).*

## Media

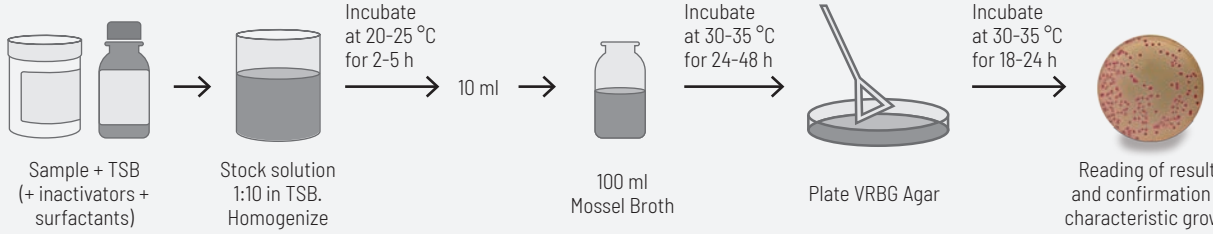
- To prepare stock solution and recovery of microorganisms: Casein Soya Bean Digest Broth (Tryptone Soy Broth)
- For selective enrichment: Mossel broth for enrichment of Enterobacteria
- For counting bacteria: Violet Red Bile Glucose Agar

| Product name | Composition (g/l) | Code | Package |
|---|---|---|---|
| **Tryptone Soy Broth (TSB) (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Papaic Digest of Soya ..... 3.0<br>D(+)-Glucose ..... 2.5<br>Digest Pancreatic of Casein ..... 17.0<br>di-Potassium Hydrogen Phosphate ..... 2.5<br>Sodium Chloride ..... 5.0<br>pH 7.3 ± 0.2 | 413820.1210 | 500 g |
| | | 413820.0914 | 5 kg |
| **Violet Red Bile Glucose Agar (VRBG) (Ph. Eur. ) (ISO21528)** | Bile Salts Mixture ..... 1.5<br>Crystal Violet ..... 0.002<br>Neutral Red ..... 0.03<br>D(+)-Glucose ..... 10.0<br>Yeast Extract ..... 3.0<br>Pancreatic Digest of Gelatine ..... 7.0<br>Sodium Chloride ..... 5.0<br>Agar ..... 15.0<br>pH 7.4 ± 0.2 | 413745.1210 | 500 g |
| | | 433745.0922 | 30 contact plates |
| | | 453745.0922 | 20 plates 90 mm |

## Method

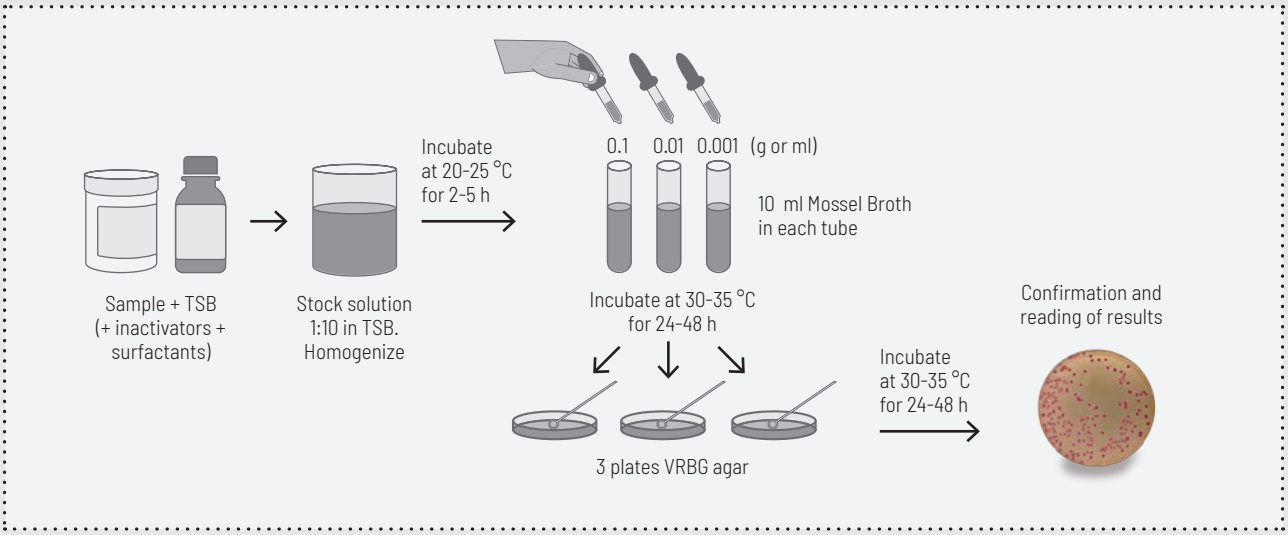
### Test for absence of Enterobacteria and other Gram-negative bacteria

1. Obtain the sample aseptically.
2. Prepare a stock solution, in a ratio of 1:10, of the sample with Casein Soya Bean Digest Broth (TSB). This solution can be supplemented with inactivators of antimicrobial agents (lecithin, sodium chloride, histidine, etc.) and surfactants that facilitate the solubility of fatty products (polysorbate 80). Homogenize.
3. Incubate at 20 - 25 °C for 2 - 5 hours.
4. Inoculate in 100 ml of Mossel Broth for enrichment of Enterobacteria with an aliquot of stock solution containing 1 g (or 1 ml) of sample.
5. Incubate at 30 - 35 °C for 24 - 48 hours.
6. Subculture on a plate of Violet Red Bile Glucose Agar.
7. Incubate at 30 - 35 °C for 18 - 24 hours.
8. Confirmation of suspicious colonies with Gram stain. The family is characterized by Gram-negative bacilli.



### Quantitative test of Enterobacteria and other Gram-negative bacteria

1. Obtain the sample aseptically.
2. Prepare a stock solution, in a ratio of 1:10, of the sample with Casein Soya Bean Digest Broth (TSB). This solution can be supplemented with inactivators of antimicrobial agents (lecithin, sodium chloride, histidine, etc.) and surfactants that facilitate the solubility of fatty products (polysorbate 80). Homogenize.
3. Incubate at 20 - 25 °C for 2 - 5 h.
4. Prepare three tubes with 10 ml of Mossel Broth for enrichment of Enterobacteria.
5. In the first tube, inoculate an aliquot of the stock solution containing 0.1 g or ml of the product to be examined. In the second tube containing 0.01 g or ml, and in the third tube containing 0.001 g or ml.
6. Incubate at 30 - 35 °C for 24 - 48 hours.
7. Subculture on a plate of Violet Red Bile Glucose Agar.
8. Incubate at 30 - 35 °C for 18 - 24 hours.
9. Growth of colonies on the medium indicates a positive result. Count of the colonies confirmed through the table (below).




| Most probable number | | | |
|---|---|---|---|
| Result for each quantity of product | | | MPN of bacteria per g or ml of product |
| 0.1 g or 0.1 ml | 0.01 g or 0.01 ml | 0.001 g or 0.001 ml | |
| + | + | + | More than 1000 |
| + | – | – | Between 1000 and 100 |
| + | – | – | Less than 100 and more than 10 |
| – | – | – | Less than 100 |

## *Escherichia coli*

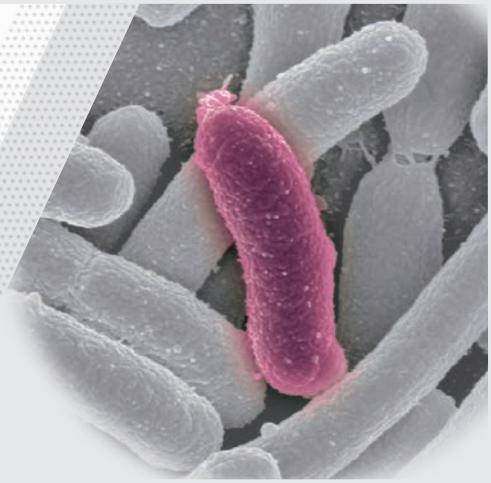
### Media and reagents

To prepare stock solution:
- Buffered Solution of Sodium Chloride-Peptone pH 7.0
- Phosphate Buffer Solution pH 7.2
- Casein Soya Bean Digest Broth (Tryptone Soy Broth)

For primary enrichment:
- Casein Soya Bean Digest Broth (Tryptone Soy Broth)

For selective enrichment:
- MacConkey Broth

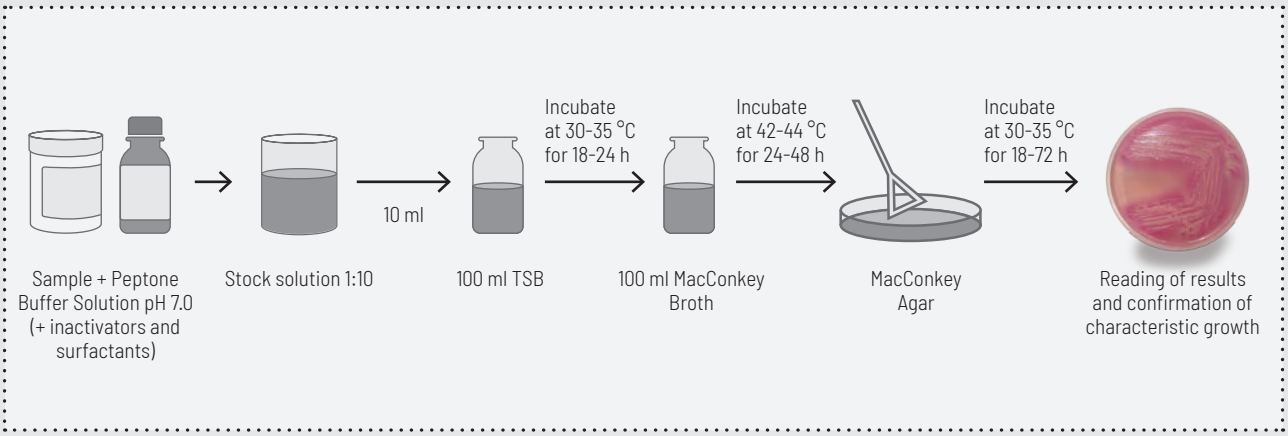
For selective isolation of *E.coli*:
- MacConkey Agar



| Product name | Composition (g/l) | Code | Package |
|---|---|---|---|
| **Buffered Sodium Chloride-Peptone solution (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Digest Pancreatic of Casein ..... 1.00<br>Potassium di-Hydrogen Phosphate ..... 3.60<br>Sodium Chloride ..... 4.30<br>di-Sodium Hydrogen Phosphate 2-hydrate ..... 7.20<br>pH 7.0 ± 0.2 | 414944.1210 | 500 g |
| | | 414944.0914 | 5 kg |
| **PBS tablets pH 7.2** (for 1 L) | Phosphate ..... 10 mM<br>NaCl ..... 140 mM<br>KCl ..... 2.7 mM | A9202,0010 | 10 tablets |
| | | A9202,0100 | 100 tablets |
| **Tryptone Soy Broth (TSB) (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Papaic Digest of Soya ..... 3.0<br>D(+)-Glucose ..... 2.5<br>Digest Pancreatic of Casein ..... 17.0<br>di-Potassium Hydrogen Phosphate ..... 2.5<br>Sodium Chloride ..... 5.0<br>pH 7.3 ± 0.2 | 413820.1210 | 500 g |
| | | 413820.0914 | 5 kg |
| **MacConkey Broth (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Ox Bile ..... 5.0<br>Lactose ..... 10.0<br>Gelatin Peptone ..... 20.0<br>Bromocresol Purple ..... 0.01<br>pH 7.3 ± 0.2 | 413780.1210 | 500 g |
| **MacConkey Agar (Ph. Eur., ISO 21567) (Dehydrated Culture Media) for microbiology** | Lactose ..... 10.0<br>Peptones (meat and casein) ..... 3.0<br>Bile Salts ..... 1.5<br>Gelatin Peptone ..... 17.0<br>Neutral Red ..... 0.03<br>Sodium Chloride ..... 5.0<br>Crystal Violet ..... 0.001<br>Agar ..... 13.5<br>pH 7.1 ± 0.2 | 413779.1210 | 500 g |

## Method

1. Obtain the sample aseptically.
2. Prepare a stock solution, in a ratio of 1:10, of sample with Buffered Solution of Sodium Chloride-Peptone pH 7.0 or similar. This buffered solution can be supplemented with inactivators of antimicrobial agents (lecithin, sodium chloride, histidine, etc.) and surfactants that facilitate the solubility of fatty products (polysorbate 80). Homogenize.
3. Seed 10 ml of the stock solution or the amount corresponding to 1 g or 1 ml of product in 100 ml of Casein Soya Bean Digest Broth (TSB) and homogenize.
4. Incubate at 30 - 35 °C for 18 - 24 hours.
5. Reseed 1 ml in 100 ml of MacConkey Broth.
6. Incubate at 42 - 44 °C for 24 - 48 hours.
7. Subculture on a MacConkey Agar plate.
8. Incubate at 30 - 35 °C for 18 - 72 hours.
9. The growth of colonies on the medium indicates the possible presence of *E. coli*.
10. Proceed with the confirmation of suspicious colonies.




## Results

The product complies with the test if no colonies are present or if the identification tests are negative.

## *Salmonella*

### Media and reagents

To prepare stock solution:
- Buffered Solution of Sodium Chloride-Peptone pH 7.0
- Phosphate Buffer Solution pH 7.2
- Casein Soya Bean Digest Broth (Tryptone Soy Broth)

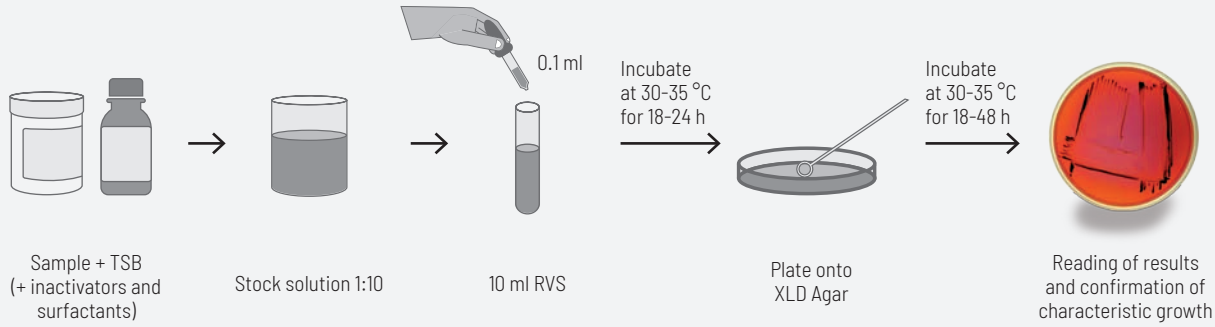
For selective enrichment:
- Rappaport-Vassiliadis broth for enrichment of *Salmonella*

For selective isolation of *Salmonella*:
- Xylose Lysine Deoxycholate Agar

| Product name | Composition (g/l) | Code | Package |
|---|---|---|---|
| **Buffered Sodium Chloride-Peptone solution (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Digest Pancreatic of Casein ..... 1.00<br>Potassium di-Hydrogen Phosphate ..... 3.60<br>Sodium Chloride ..... 4.30<br>di-Sodium Hydrogen Phosphate 2-hydrate ..... 7.20<br>pH 7.0 ± 0.2 | 414944.1210 | 500 g |
| | | 414944.0914 | 5 kg |
| **PBS tablets pH 7.2** (for 1 L) | Phosphate ..... 10 mM<br>NaCl ..... 140 mM<br>KCl ..... 2.7 mM | A9202,0010 | 10 tablets |
| | | A9202,0100 | 100 tablets |
| **Tryptone Soy Broth (TSB) (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Papaic Digest of Soya ..... 3.0<br>D(+)-Glucose ..... 2.5<br>Digest Pancreatic of Casein ..... 17.0<br>di-Potassium Hydrogen Phosphate ..... 2.5<br>Sodium Chloride ..... 5.0<br>pH 7.3 ± 0.2 | 413820.1210 | 500 g |
| | | 413820.0914 | 5 kg |
| **XLD Medium (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Ammonium Iron(III) Citrate ..... 0.8<br>Yeast Extract ..... 3.0<br>Lactose ..... 7.5<br>L-Lysine ..... 5.0<br>Phenol Red ..... 0.08<br>Saccharose ..... 7.5<br>Sodium Chloride ..... 5.0<br>Sodium Deoxycholate ..... 2.5<br>Sodium Thiosulfate ..... 6.8<br>D(+)-Xylose ..... 3.5<br>Agar ..... 13.5<br>pH 7.4 ± 0.2 | 413826.1210 | 500 g |

## Method

1. Obtain the sample aseptically.
2. Prepare a stock solution, in a ratio of 1:10, using not less than 10 g or 10 ml of sample with Casein Soya Bean Digest Broth (TSB). This broth can be supplemented with inactivators of antimicrobial agents (lecithin, sodium chloride, histidine, etc.) and surfactants that facilitate the solubility of fatty products (polysorbate 80). Homogenize.
3. Incubate at 30 - 35 °C for 18 - 24 hours.
4. Transfer 0.1 ml of casein soya bean digest broth (TSB) to 10 ml of Rappaport Vassiliadis *Salmonella* enrichment broth and incubate at 30 - 35 °C for 18 - 24 hours.
5. Subculture on a plate of Xylose Lysine Deoxycholate Agar.
6. Incubate at 30 – 35 ° C for 18 - 48 hours.
7. Those red colonies with or without a black center are considered suspicious of *Salmonella*.
8. All suspicious colonies will be confirmed with identification tests.




## Results

The product complies with the test if no colonies are present or if the identification tests are negative.

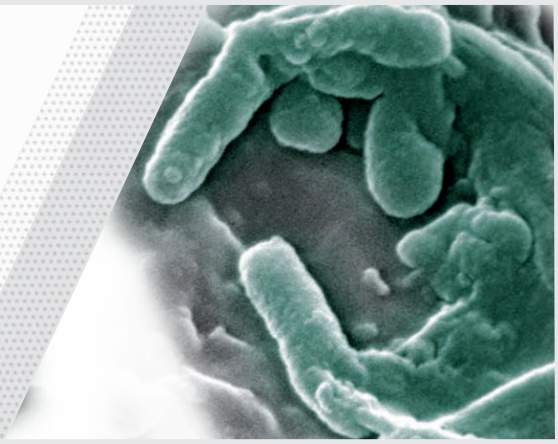
## *Pseudomonas aeruginosa*

**Media and reagents**

To prepare stock solution:
- Buffered Solution of Sodium Chloride-Peptone pH 7.0
- Phosphate Buffer Solution pH 7.2
- Casein Soya Bean Digest Broth (Tryptone Soy Broth)

For primary enrichment:
- Casein Soya Bean Digest Broth (TSB)

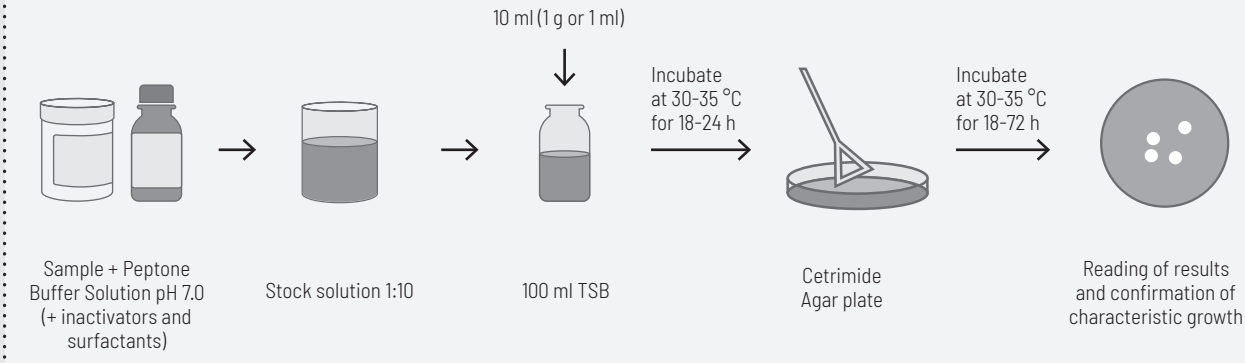
For selective isolation of *Pseudomonas aeruginosa*:
- Cetrimide Agar



| Product name | Composition (g/l) | Code | Package |
|---|---|---|---|
| **Buffered Sodium Chloride-Peptone solution (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Digest Pancreatic of Casein ..... 1.00<br>Potassium di-Hydrogen Phosphate ..... 3.60<br>Sodium Chloride ..... 4.30<br>di-Sodium Hydrogen Phosphate 2-hydrate ..... 7.20<br>pH 7.0 ± 0.2 | 414944.1210 | 500 g |
| | | 414944.0914 | 5 kg |
| **PBS tablets pH 7.2** (for 1 L) | Phosphate ..... 10 mM<br>NaCl ..... 140 mM<br>KCl ..... 2.7 mM | A9202,0010 | 10 tablets |
| | | A9202,0100 | 100 tablets |
| **Tryptone Soy Broth (TSB) (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Papaic Digest of Soya ..... 3.0<br>D(+)-Glucose ..... 2.5<br>Digest Pancreatic of Casein ..... 17.0<br>di-Potassium Hydrogen Phosphate ..... 2.5<br>Sodium Chloride ..... 5.0<br>pH 7.3 ± 0.2 | 413820.1210 | 500 g |
| | | 413820.0914 | 5 kg |
| **Cetrimide Agar (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Cetrimide ..... 0.3<br>Magnesium Chloride ..... 1.4<br>Pancreatic Digest of Gelatine ..... 20.0<br>Potassium Sulfate ..... 10.0<br>Agar ..... 13.6<br>pH 7.2 ± 0.2 | 416256.1210 | 500 g |
| | | 456256.0922 | 20 plates 90 mm |

## Method

1. Obtain the sample aseptically.
2. Prepare a stock solution, in a ratio of 1:10, of sample with Buffered Solution of Sodium Chloride-Peptone pH 7.0 or similar. This buffered solution can be supplemented with inactivators of antimicrobial agents (lecithin, sodium chloride, histidine, etc.) and surfactants that facilitate the solubility of fatty products (polysorbate 80). Homogenize.
3. Seed 10 ml of the stock solution or the amount corresponding to 1 g or 1 ml of product in 100 ml of Casein Soya Bean Digest Broth (TSB) and homogenize.
4. Incubate at 30 - 35 °C for 18 - 24 hours.
5. Subculture on a Cetrimide agar plate.
6. Incubate at 30 - 35 °C for 18 - 72 hours.
7. The growth of colonies on the medium indicates the possible presence of *P. aeruginosa*.
8. Proceed with the confirmation of suspicious colonies.




## Results

The product complies with the test if no colonies are present or if the identification tests are negative.

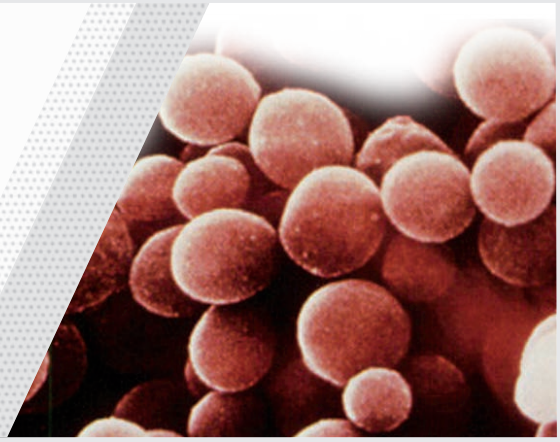
## *Staphylococcus aureus*

**Media and reagents**

To prepare stock solution:
- Buffered Solution of Sodium Chloride-Peptone pH 7.0
- Phosphate Buffer Solution pH 7.2
- Casein Soya Bean Digest Broth (Tryptone Soy Broth)

For primary enrichment:
- Casein Soya Bean Digest Broth (TSB)

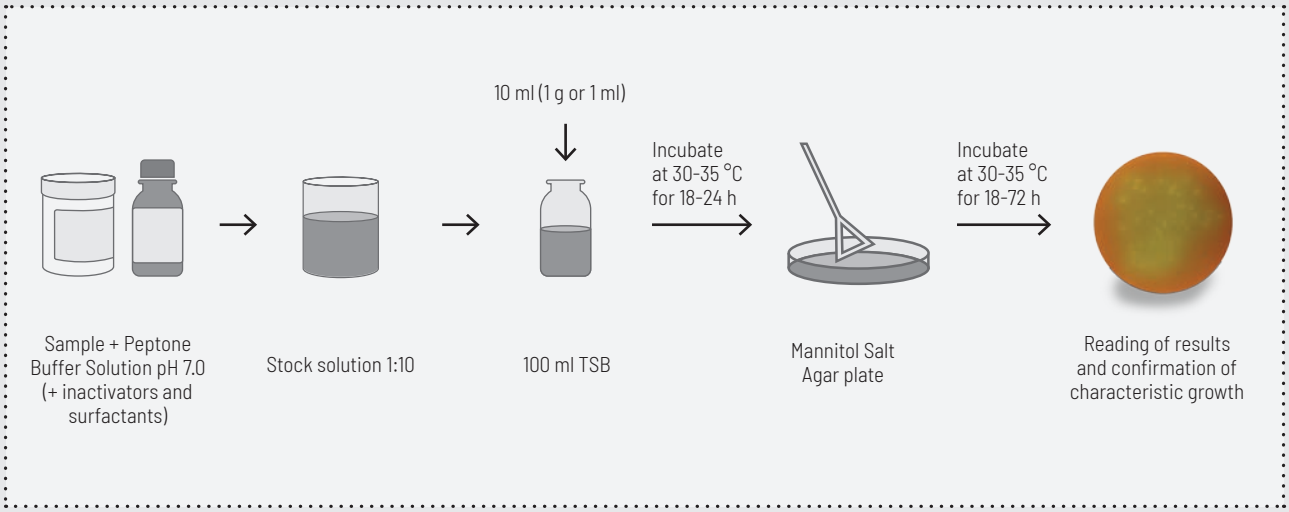
For selective isolation of *Staphylococcus aureus*:
- Salt and Mannitol, Agar



| Product name | Composition (g/l) | Code | Package |
|---|---|---|---|
| **Buffered Sodium Chloride-Peptone solution (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Digest Pancreatic of Casein ..... 1.00<br>Potassium di-Hydrogen Phosphate ..... 3.60<br>Sodium Chloride ..... 4.30<br>di-Sodium Hydrogen Phosphate 2-hydrate ..... 7.20<br>pH 7.0 ± 0.2 | 414944.1210 | 500 g |
| | | 414944.0914 | 5 kg |
| **PBS tablets pH 7.2** (for 1 L) | Phosphate ..... 10 mM<br>NaCl ..... 140 mM<br>KCl ..... 2.7 mM | A9202,0010 | 10 tablets |
| | | A9202,0100 | 100 tablets |
| **Tryptone Soy Broth (TSB) (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Papaic Digest of Soya ..... 3.0<br>D(+)-Glucose ..... 2.5<br>Digest Pancreatic of Casein ..... 17.0<br>di-Potassium Hydrogen Phosphate ..... 2.5<br>Sodium Chloride ..... 5.0<br>pH 7.3 ± 0.2 | 413820.1210 | 500 g |
| | | 413820.0914 | 5 kg |
| **Mannitol Salt Agar (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Sodium Chloride ..... 75.0<br>D(-)-Mannitol ..... 10.0<br>Meat Extract ..... 1.0<br>Digest Pancreatic of Casein ..... 5.0<br>Peptic Digest of Animal Tissue ..... 5.0<br>Phenol Red ..... 0.025<br>Agar ..... 15.0<br>pH 7.4 ± 0.2 | 413783.1210 | 500 g |

## Method

1. Obtain the sample aseptically.
2. Prepare a stock solution, in a ratio of 1:10, of sample with peptone buffered solution at pH 7.0, Casein Soya Bean Digest Broth (TSB) or a buffered phosphate solution of pH 7.2. This buffered solution can be supplement with inactivators of antimicrobial agents (lecithin, sodium chloride, histidine, etc.) and surfactants that facilitate the solubility of fatty products (polysorbate 80). Homogenize.
3. Seed 10 ml of the stock solution or the amount corresponding to 1 g or 1 ml of product in 100 ml of Casein Soya Bean Digest Broth (TSB) and homogenize.
4. Incubate at 30 - 35 °C for 18 - 24 hours.
5. Subculture on a plate of Salt and Mannitol Agar.
6. Incubate at 30 – 35 °C for 18 - 72 hours.
7. The yellow-white colonies with yellow halo are suspicious of being *Staphylococcus aureus.*
8. Confirmation of suspicious colonies with biochemical tests such as the coagulase test and the deoxyribonuclease. *Staphylococcus aureus* are positive for both tests.




## Results

The product complies with the test if no colonies are present or if the identification tests are negative.

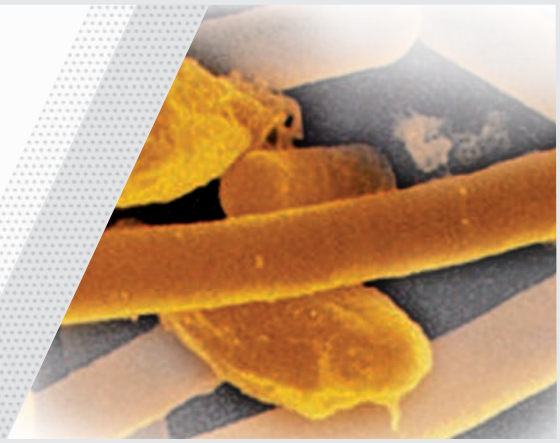
## *Clostridia*

**Media and reagents**

To prepare stock solution:
- Buffered Solution of Sodium Chloride-Peptone pH 7.0
- Phosphate Buffer Solution pH 7.2
- Casein Soya Bean Digest Broth (Tryptone Soy Broth)

For selective enrichment:
- Reinforced Media for *Clostridia*

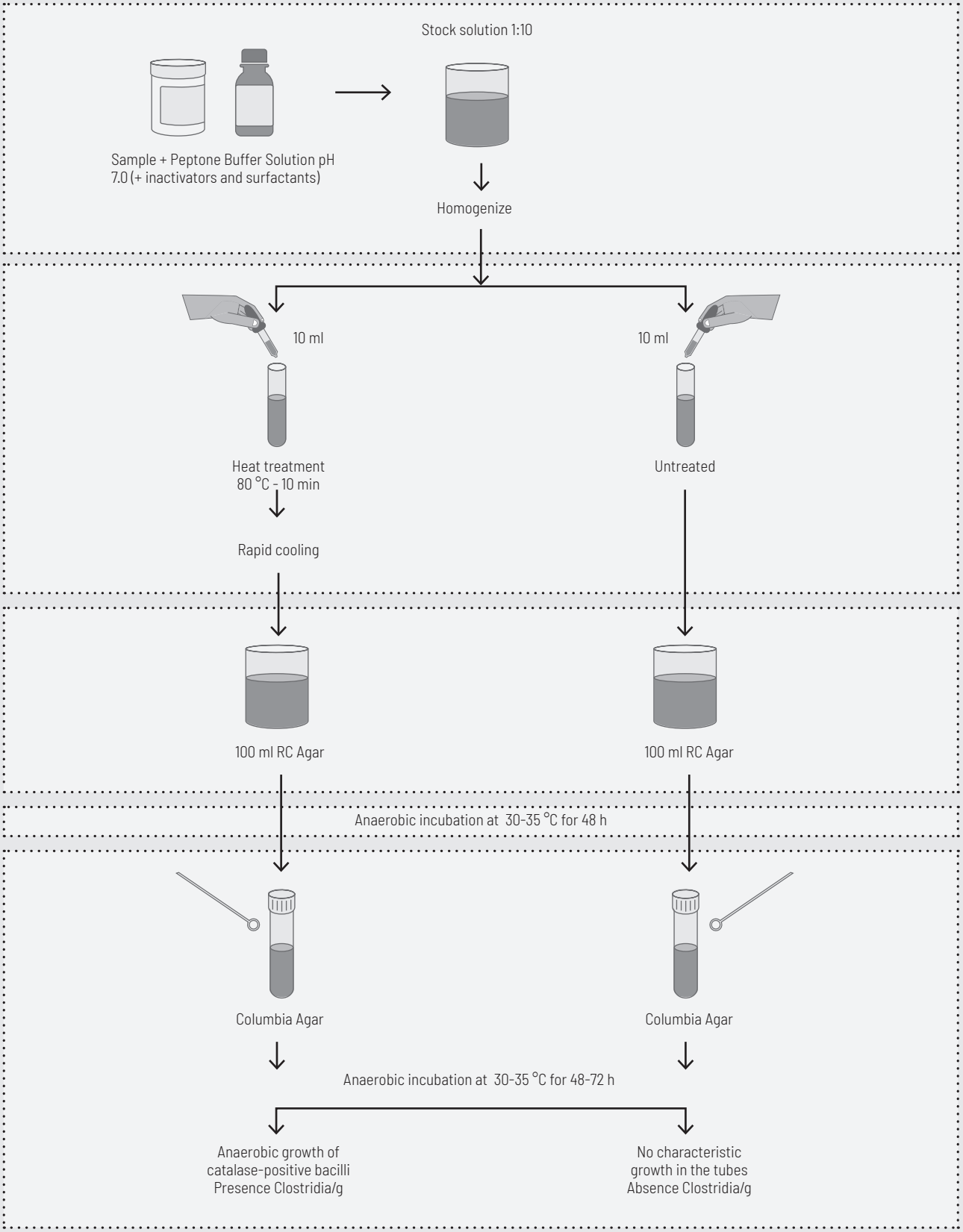
For selective isolation of *Clostridia*:
- Columbia Agar



| Product name | Composition (g/l) | Code | Package |
|---|---|---|---|
| **Buffered Sodium Chloride-Peptone solution (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Digest Pancreatic of Casein ..... 1.00<br>Potassium di-Hydrogen Phosphate ..... 3.60<br>Sodium Chloride ..... 4.30<br>di-Sodium Hydrogen Phosphate 2-hydrate ..... 7.20<br>pH 7.0 ± 0.2 | 414944.1210 | 500 g |
| | | 414944.0914 | 5 kg |
| **PBS tablets pH 7.2** (for 1 L) | Phosphate ..... 10 mM<br>NaCl ..... 140 mM<br>KCl ..... 2.7 mM | A9202,0010 | 10 tablets |
| | | A9202,0100 | 100 tablets |
| **Tryptone Soy Broth (TSB) (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Papaic Digest of Soya ..... 3.0<br>D(+)-Glucose ..... 2.5<br>Digest Pancreatic of Casein ..... 17.0<br>di-Potassium Hydrogen Phosphate ..... 2.5<br>Sodium Chloride ..... 5.0<br>pH 7.3 ± 0.2 | 413820.1210 | 500 g |
| | | 413820.0914 | 5 kg |

### Method

1. Obtain the sample aseptically.
2. Prepare a stock solution, in a ratio of 1:10 (with a minimum total volume of 20 ml), using not less than 2 g or 2 ml of sample with peptone buffered solution at pH 7.0, Casein Soya Bean Digest Broth (TSB) or a buffered phosphate solution of pH 7.2. This buffered solution can be supplement with inactivators of antimicrobial agents (lecithin, sodium chloride, histidine, etc.) and surfactants that facilitate the solubility of fatty products (polysorbate 80). Homogenize.
3. Divide the sample into 2 portions of at least 10 ml.
4. One of the portions is heat treated at 80 °C for 10 min and then cooled rapidly. The second portion does not undergo any treatment.
5. Seed, separately, 10 ml of each of the portions (treated and untreated) in 100 ml containers of Reinforced for Clostridia medium.
6. Anaerobic incubation at 30 - 35 °C for 48 hours.
7. Subculture, separately, the two samples on Columbia Agar medium.
8. Anaerobic incubation at 30 - 35 °C for 48-72 hours.
9. Observation of results: The anaerobic growth of bacilli (with or without endospores) that give negative catalase indicates the presence of *Clostridia*.


Stock solution 1:10
Sample + Peptone Buffer Solution pH 7.0 (+ inactivators and surfactants)
Homogenize
10 ml
10 ml
Heat treatment 80 °C - 10 min
Untreated
Rapid cooling
100 ml RC Agar
100 ml RC Agar
Anaerobic incubation at 30-35 °C for 48 h
Columbia Agar
Columbia Agar
Anaerobic incubation at 30-35 °C for 48-72 h
Anaerobic growth of catalase-positive bacilli Presence Clostridia/g
No characteristic growth in the tubes Absence Clostridia/g

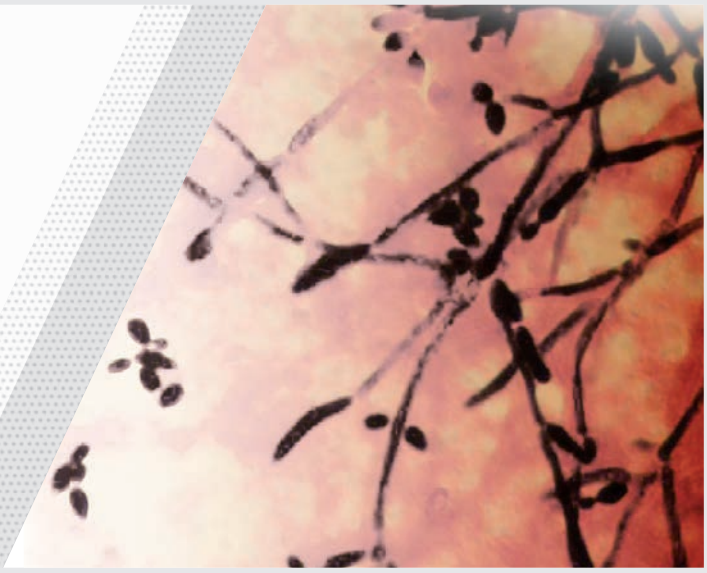
## *Candida albicans*

### Media and reagents

To prepare stock solution:
- Buffered Solution of Sodium Chloride-Peptone pH 7.0
- Phosphate Buffer Solution pH 7.2
- Casein Soya Bean Digest Broth (Tryptone Soy Broth)

For primary enrichment:
- Sabouraud Dextrose Broth (Sabouraud Glucose Broth)

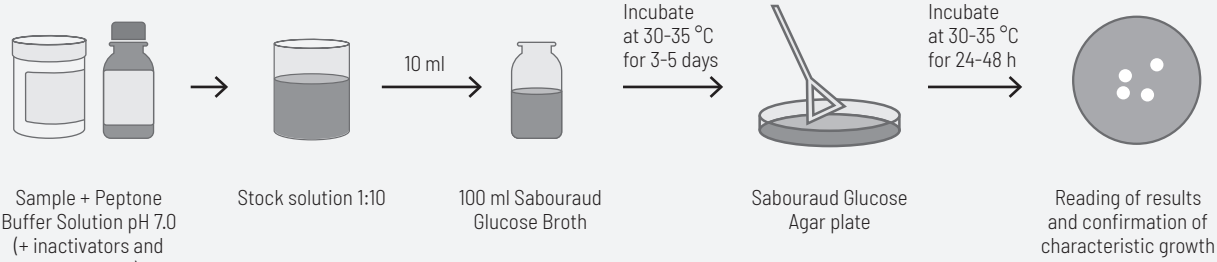
For isolation of *Candida albicans*:
- Sabouraud Dextrose Agar (Sabouraud Glucose Agar)



| Product name | Composition (g/l) | Code | Package |
|---|---|---|---|
| **Buffered Sodium Chloride-Peptone solution (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Digest Pancreatic of Casein ..... 1.00<br>Potassium di-Hydrogen Phosphate ..... 3.60<br>Sodium Chloride ..... 4.30<br>di-Sodium Hydrogen Phosphate 2-hydrate ..... 7.20<br>pH 7.0 ± 0.2 | 414944.1210 | 500 g |
| | | 414944.0914 | 5 kg |
| **PBS tablets pH 7.2** (for 1 L) | Phosphate ..... 10 mM<br>NaCl ..... 140 mM<br>KCl ..... 2.7 mM | A9202,0010 | 10 tablets |
| | | A9202,0100 | 100 tablets |
| **Tryptone Soy Broth (TSB) (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | Papaic Digest of Soya ..... 3.0<br>D(+)-Glucose ..... 2.5<br>Digest Pancreatic of Casein ..... 17.0<br>di-Potassium Hydrogen Phosphate ..... 2.5<br>Sodium Chloride ..... 5.0<br>pH 7.3 ± 0.2 | 413820.1210 | 500 g |
| | | 413820.0914 | 5 kg |
| **Sabouraud Glucose Broth (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | D(+)-Glucose ..... 20.0<br>Mixture of Peptic Digest of Animal Tissue and Pancreatic Digest of Casein (1:1) ..... 10.0<br>pH 5.6 ± 0.2 | 413804.1210 | 500 g |
| **Sabouraud Glucose Agar (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | D(+)-Glucose ..... 40.0<br>Mixture of Peptic Digest of Animal Tissue and Pancreatic Digest of Casein (1:1) ..... 10.0<br>Agar ..... 15.0<br>pH 5.6 ± 0.2 | 413802.1210 | 500 g |
| | | 453802.0922 | 20 plates 90 mm |
| **Sabouraud Glucose Agar+Chloramphenicol (Ph. Eur.) (Dehydrated Culture Media) for microbiology** | D(+)-Glucose ..... 40.0<br>Chloramphenicol ..... 0.05<br>Mixture of Peptones ..... 10.0<br>Agar ..... 15.0<br>pH 5.6 ± 0.2 | 413842.1210 | 500 g |
| | | 433842.0922 | 30 contact plates |

## Method

1. Obtain the sample aseptically.
2. Prepare a stock solution, in a ratio of 1:10, of sample with peptone buffered solution at pH 7.0, Casein Soya Bean Digest Broth (TSB) or a buffered phosphate solution of pH 7.2. This buffered solution can be supplement with inactivators of antimicrobial agents (lecithin, sodium chloride, histidine, etc.) and surfactants that facilitate the solubility of fatty products (polysorbate 80). Homogenize.
3. Seed 10 ml of the stock solution or the amount corresponding to 1 g or 1 ml of product in 100 ml of Sabouraud Dextrose Broth and homogenize.
4. Incubate at 30 - 35 °C for 3 to 5 days.
5. Subculture on Sabouraud Glucose Agar plate.
6. Incubate at 30 – 35 °C for 24-48 hours.
7. The growth of white colonies may indicate the presence of *Candida albicans*.
8. Confirmation of suspicious colonies with identification tests. The product complies if the confirmatory identification tests are negative.




## Results

The product complies with the test if no colonies are present or if the identification tests are negative.

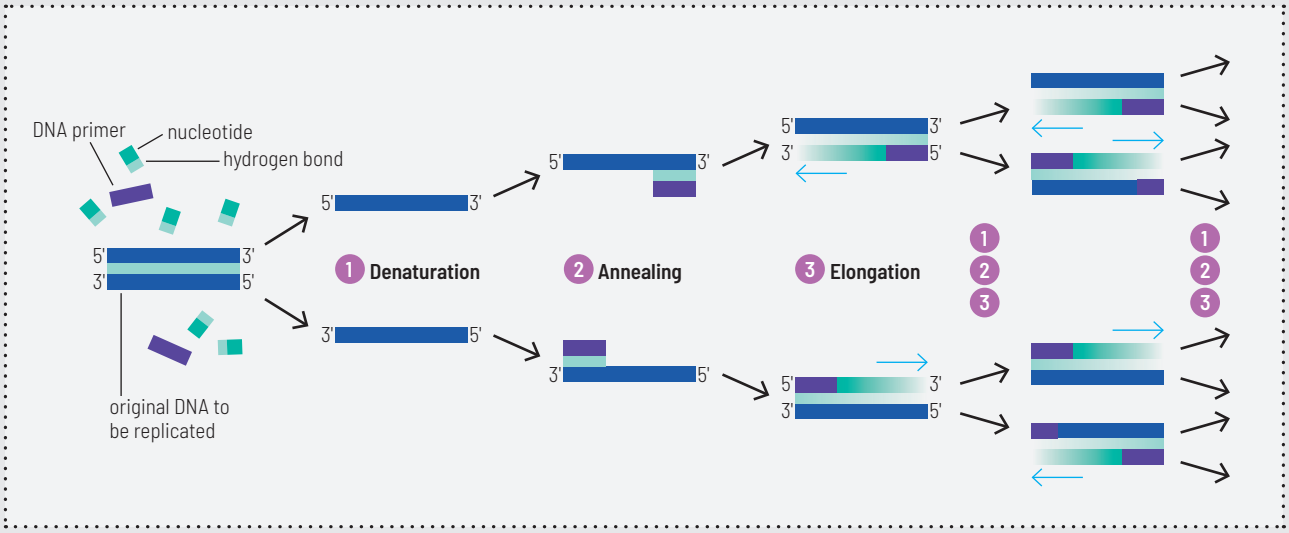
# Nucleic acid amplification techniques

Nucleic acid amplification techniques are based on two approaches:

1. Amplification via PCR, ligase chain reaction (LCR), or isothermal ribonucleic acid (RNA) amplification
2. Amplification of a hybridisation signal

We will focus only on the PCR method.

**The Polymerase Chain Reaction (PCR)** is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.




PCR is a procedure that allows specific in vitro amplification of segments of DNA or RNA after reverse transcription into cDNA. The double-stranded DNA is denatured into single-stranded DNA, two synthetic oligonucleotide primers are attached one on each side but on different strands of the DNA. The short double strands serve as a starting position for the DNA polymerase to amplify this region. This occurs in cycles consisting of:

1. Heat denaturation of the template into two single strands by breaking the hydrogen bonds
2. Specific annealing of the primers under the right experimental conditions
3. Extension of the primers via DNA polymerase and nucleotides in the assay

So, in every cycle, the number of amplicons is doubled in an exponential way.

PanReac AppliChem offers enzymes for the PCR at high performance.

## Enzymes for PCR

| Product name | Code | Package |
|---|---|---|
| **SuperHot Taq DNA Polymerase** | A5231,0200 | 200 U |
| **Taq DNA Polymerase** | A5186,0500 | 500 U |
| **Taq DNA Polymerase DNA-free** | A5434,0500 | 500 U |

## Test Material and Prevention of contamination

All sample handling processes such as sampling, transport and storage, should be carried out under conditions that minimize degradation. Especially when working with RNA, precautions are necessary since it is highly sensitive against ribonucleases.

Also some reagents added to the experiment as anticoagulants or preservatives may interfere with the experimental procedures.

Because PCR provides high sensitivity, samples must be protected against external contamination with unwanted sequences.

First, the working areas should be strictly separated and kept clean. But this alone is not always sufficient.

Because of this, PanReac AppliChem has developed a solution by supporting your work with the DNA Exitus Plus™ technology, not only avoiding contamination but also being able to decontaminate your workspace, pipettes, machines, etc.

## DNA-ExitusPlus™ technology

DNA-ExitusPlus™ is a patented reagent for the removal of nucleic acid contamination from laboratory surfaces and equipment. The solution employs a **mild** and **non-corrosive** chemistry for a **rapid** nonenzymatic **degradation of nucleic acids.** Already, short incubation times with DNA-ExitusPlus™ completely remove unwanted DNA and RNA from work surfaces and tools.

There are two different versions of DNA-ExitusPlus™ available:

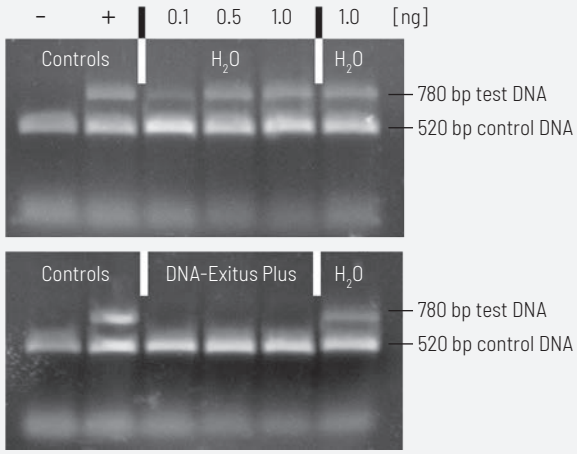
- DNA-ExitusPlus™ (A7089) includes a color indicator to easily visualize the surface area covered with the reagent.
- DNA-ExitusPlus™ IF (A7409) is almost without color.

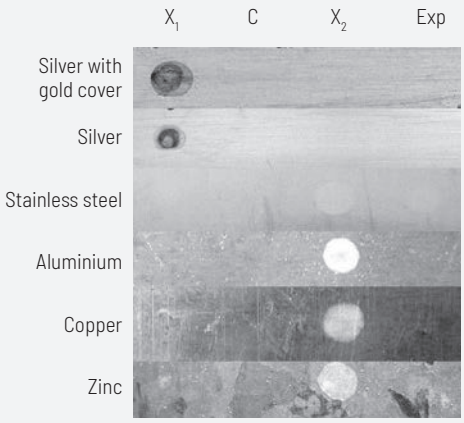
Both solutions darken with time due to redox-active components contained in the solutions.

*Note: There are no differences in the application protocols of DNA-ExitusPlus™ and DNA-ExitusPlus™ IF. Therefore we do not name the IF form in the following characteristics and procedures.*

**The unique characteristics of DNA-ExitusPlus™**

- Catalytic and cooperative effects of the components cause a very rapid non-enzymatic, non-sequence-specific degradation of **DNA** and **RNA** molecules.
- All components of DNA-ExitusPlus™ are readily **bio-degradable** and **not harmful** nor toxic for humans.
- No aggressive mineral acids or alkaline substances are used.
- Equipment and materials are not damaged or corroded even after prolonged incubation times.
- No toxic fumes.
- Elevated temperatures above approx. 50 °C speed up the reaction and the activity.




**Complete removal of DNA contaminations by DNA ExitusPlus™ determined by sensitive PCR assay.**

Experimental procedure:

Test DNA (0.1 to 1 ng) was lyophilized on the inner surface of PCR tubes, incubated for 20 seconds with sterile water or DNA-ExitusPlus™, then washed twice with 100 µl of sterile water.

For the PCR test we used 50 µl of each of the reaction mixtures, containing the appropriate primers for the amplification of the control and test DNA sequences. Control DNA (1 ng) in each sample proves that the PCR reaction is not inhibited.

Results: Amplification of a DNA band, corresponding to the test DNA, indicates that intact DNA molecules are present. Conversely, if no amplification DNA bands are present, it indicates complete degradation of the test DNA. The negative control with sterile water ($H_2O$) exhibits DNA bands for the test and control templates whilst after treatment with DNA-ExitusPlus™ only the fragment of the control DNA is amplified.

**DNA-ExitusPlus™ has no corrosive potential compared to conventional DNA decontamination reagents.**

Metal plates representing typical laboratory materials and equipment were treated with 10 µl of each indicated reagent for 20 minutes. No corrosive effects were observed when using DNA-ExitusPlus™ (in some cases one observes a polishing effect by the removal of dirt or oxide layers).

C = water

Exp = DNA-ExitusPlus™

X1, X2 = other commercially available products

## DNA-ExitusPlus™ products

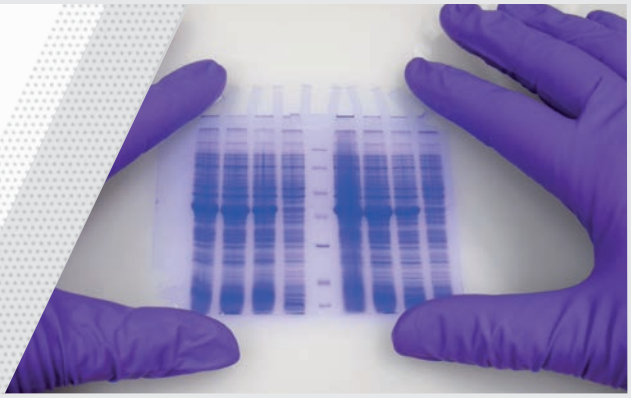
- Not toxic for humans
- Does not harm material
- Easy to use
- Very effective


| Product name | Code | Package |
|---|---|---|
| **Autoclave-ExitusPlus™** | A7600,1000 | 6 x 1 L |
| **DNA-ExitusPlus™** | A7089,0100 | 100 ml |
| | A7089,0500 | 500 ml |
| | A7089,1000RF | 1 L refill |
| | A7089,2500RF | 2.5 L refill |
| **DNA-ExitusPlus™ IF** | A7409,0100 | 100 ml |
| | A7409,0500 | 500 ml |
| | A7409,1000RF | 1 L refill |
| | A7409,2500RF | 2.5 L refill |
| | A7409,5000 | 5 L |
| **ExitusPlus™ Activity Test** | A9411,0025 | 25 strips |
| **RNase-ExitusPlus™** | A7153,0500 | 500 ml |
| | A7153,1000RF | 1 L refill |
| | A7153,2500RF | 2.5 L refill |

## Detection of Nucleic Acids

PCR amplicons or other genetic material might be identified by different methods and parameters. Detection and characterization by size is one of the most important methods. Mostly agarose gels are used for this purpose.

PanReac Applichem serves a fine selection of agaroses covering most standard applications.



### Agaroses

| Product name | Gel strength 1 % | Gel strength 1.5 % | EEO | Code | Package |
|---|---|---|---|---|---|
| **Agarose MP** | ≥ 1800 g/cm² | ≥ 3200 g/cm² | ≤ 0.12 | A1091,0100 | 100 g |
| | | | | A1091,0250 | 250 g |
| | | | | A1091,0500 | 500 g |
| **Agarose low EEO (Agarose Standard)** | ≥ 1200 g/cm² | ≥ 2300 g/cm² | 0.09 - 0.13 | A2114,0100 | 100 g |
| | | | | A2114,0250 | 250 g |
| | | | | A2114,0500 | 500 g |
| **Agarose medium EEO** | ≥ 750 g/cm² | ≥ 2000 g/cm² | 0.16 - 0.19 | A2116,0100 | 100 g |
| | | | | A2116,0500 | 500 g |
| **Agarose Basic** | ≥ 1000 g/cm² | ≥ 2200 g/cm² | 0.14 - 0.16 | A8963,0100 | 100 g |
| | | | | A8963,0250 | 250 g |
| | | | | A8963,0500 | 500 g |
| | | | | A8963,1000 | 1 kg |

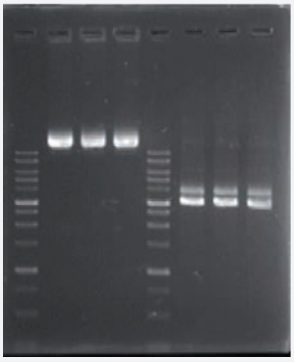
### DNA-Dye NonTox. The perfect alternative to Ethidium Bromide

DNA has to be made visible. The standard routine is to use ethidium bromide which is the most widely used DNA stain in molecular biology. However, due to safety and health concerns associated with exposure to this chemical, there has been increased interest in the use of alternative DNA stains.

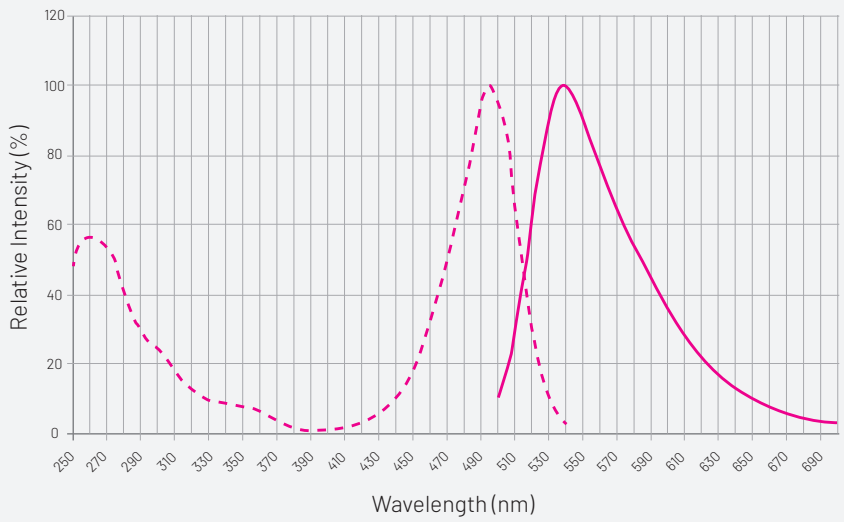
DNA-Dye NonTox is a non-toxic fluorescent reagent supplied in loading buffer, being a highly sensitive dye for the staining of DNA in gel electrophoresis. The dye produces instant visualization of DNA bands on gels upon blue light or UV illumination.

- As **sensitive** as Ethidium Bromide.
- **Non-Hazardous**, non-mutagenic and with low toxicity.
- **Low environmental impact.** No need of special measures with respect to waste management.
- **DNA structure and integrity not affected**, so higher transformation rates are achieved.
- DNA-Dye NonTox does **not intercalate**, therefore, no variation in the migration behaviour is observed.

DNA-Dye NonTox is supplied as a ready-to-use 6X loading dye, containing three tracking dyes: bromophenol blue, xylene cyanol and orange G. Since the loading dye is added directly to the sample, there is only a very low background staining observed. After the gel run, no destaining is required.


**Agarose gel electrophoresis of DNA stained with DNA-Dye NonTox.** DNA marker (M) and samples (1 -6) were stained with DNA-Dye NonTox, separated by agarose gel electrophoresis and subsequently detected under UV light.




Fluorescence excitation/ emission **spectra of DNA-Dye NonTox** nucleic acid gel stain bound to DNA.

Besides the agarose and visualization substances for assays with DNA/ RNA you will need further material like Buffers, Markers and Special solutions.

## Buffers for nucleic acids

| Product name | Code | Package |
|---|---|---|
| **CTAB - Lysis buffer BioChemica** | A4150,0500 | 500 ml |
| | A4150,1000 | 1 L |
| **SSC buffer (20X) for molecular biology** | A1396,1000 | 1 L |
| **TAE buffer (50X) for molecular biology** | A4686,1000 | 1 L |
| **TAE buffer (50X)** | A1691,0500 | 500 ml |
| | A1691,1000 | 1 L |
| **TAE buffer (10X) for molecular biology** | A4227,1000 | 1 L |
| | A4227,5000 | 5 L |
| **TBE buffer (10X) for molecular biology** | A3945,1000 | 1 L |
| **TBE buffer (10X)** | A0972,1000 | 1 L |
| | A0972,5000PE | 5 L |
| | A0972,9010CT | 10 L |
| | A0972,9010PE | 10 L |
| **TBE buffer (10X) powder** | A4348,1000 | 1 L |
| | A4348,5000 | 5 L |
| | A4348,9010 | 10 L |
| **TBE buffer (5X) for molecular biology** | A4228,5000PE | 5 L |
| **TBE buffer (5X)** | A1417,5000PE | 5 L |
| **TBE buffer (5X) powder** | A4394,9010 | 10 L |
| **TE buffer (100X) pH 8.0** | A0973,0500 | 500 ml |
| **TE buffer (1X) pH 7.5** | A3837,1000 | 1 L |
| **TE buffer (1X) pH 8.0** | A2575,1000 | 1 L |
| **TE buffer (1X) pH 8.0 for molecular biology** | A0386,0500 | 500 ml |
| | A0386,1000 | 1 L |
| **TE buffer (1X) pH 8.0 low EDTA for molecular biology** | A8569,0500 | 500 ml |
| | A8569,1000 | 1 L |



## Ladders and Markers

| Product name | Code | Package |
|---|---|---|
| **DNA Ladder 50 bp** | A8368,0050 | 50 µg |
| **DNA Ladder 100 bp** | A5191,0005 | 50 µg |
| | A5191,0025 | 250 µg |
| **DNA Ladder 100 bp (lyophilised)** | A3470,0050 | 50 µg |
| **DNA Ladder 100 bp (plus)** | A5216,0005 | 50 µg |
| | A5216,0025 | 250 µg |
| **DNA Ladder 1 kb** | A5207,0005 | 50 µg |
| | A5207,0025 | 250 µg |
| **DNA Ladder Mix 100 - 5000 (lyophilised)** | A3660,0050 | 50 µg |
| **DNA Marker Phage Lambda - Sty I** | A5194,0005 | 50 µg |

DNA Ladder 1 kb

| Lenght [bp] | Quantity [ng] |
|---|---|
| 10000 | 64 |
| 8000 | 66 |
| 6000 | 79 |
| 5000 | 94 |
| 4000 | 79 |
| 3000 (x2) | 224 |
| 2500 | 94 |
| 2000 | 74 |
| 1500 | 56 |
| 1000 (x2) | 113 |
| 750 | 28 |
| 500 | 19 |
| 250 | 10 |

## Evaluation and interpretation of results. Quality assurance

Valid results for work with genetic material can only be obtained if positive and negative controls are used, especially due to the high sensitivity of the PCR and the risk of contamination. A double or triple test may also be performed to ensure the validity of the experimental result.

PCR assay systems need to be validated. For this, we offer the PCR Cycler Validation Kit.

### PCR Cycler Validation Kit, code A9742,0002

Validation of the PCR Cycler using standardized thermosensitive PCR protocols.

Non-specific or false-negative PCR results are a serious challenge and might be caused due to a defective heating element of the PCR cycler. Therefore, routine control of the reaction temperature is crucial to detect any irregularities in time, and to avoid bad amplifications. PanReac AppliChem's PCR Cycler Validation Kit is a reliable and reproducible test system for fast evaluation of the thermocycler's heating elements.

- PCR Cycler Validation Kit is **fast, convenient, easy** to perform
- Does **not require any special equipment** such as thermo sensors, software or instruments
- Suitable **for all type of thermal cyclers**, regardless of manufacturer and model.

For the validation of qPCR thermal cyclers we offer a modified version based on the same test principle: the qPCR Cycler Validation Kit, code A9801. Both kits are applicable with any block PCR/qPCR cycler in research or industrial quality assurance laboratories in order to meet the legal requirements for reliability testing of instruments used for analysis (ISO 17025, EN 45001, ISO 13485, GLP, GMP).

# Microbiological assay of antibiotics

Pharmacopoeias describe methods for measuring antibiotic potency.

The allowed methods are the Diffusion Method or the Turbidimetric Method. The first is conducted on plates or petri dishes, the second in solutions. Reference substances (standards), solvents and reagents according to the pharmacopoeia are required to perform the test, either by the diffusion method or by the turbidimetric method.

**Solvents and reagents to prepare solutions of the reference substance and the antibiotic to be examined**

| Product name | Code | Package |
|---|---|---|
| **N,N-Dimethylformamide (Reag. Ph. Eur.) for analysis, ACS, ISO** | 131785.1611 | 1000 ml |
| | 131785.1612 | 2.5 L |
| | 131785.1214 | 5 L |
| | 131785.0716 | 25 L |
| **Dimethyl Sulfoxide (Reag. Ph. Eur.) for analysis, ACS** | 131954.1611 | 1000 ml |
| | 131954.1612 | 2.5 L |
| | 131954.1214 | 5 L |
| | 131954.0715 | 10 L |
| **Ethanol 96% v/v (USP, BP, Ph.Eur.) pure, pharma grade** | 141085.1211 | 1000 ml |
| | 141085.1212 | 2.5 L |
| | 141085.1214 | 5 L |
| | 141085.1315 | 10 L |
| **Formaldehyde 37-38% w/w stabilized with methanol (USP, BP, Ph. Eur.) pure, pharma grade** | 141328.1211 | 1000 ml |
| | 141328.1212 | 2.5 L |
| | 141328.1214 | 5 L |
| **Hydrochloric Acid 0.01 mol/l (0.01N) volumetric solution** | 182884.1211 | 1000 ml |
| **Hydrochloric Acid 0.1 mol/l (0.1N) volumetric solution** | 181023.1211 | 1000 ml |
| | 181023.1212 | 2.5 L |
| | 181023.1214 | 5 L |
| | 181023.0715 | 10 L |
| | 181023.1315 | 10 L |
| **Methanol (Reag. Ph. Eur.) for analysis, ACS, ISO** | 131091.1211 | 1000 ml |
| | 131091.1611 | 1000 ml |
| | 131091.1212 | 2.5 L |
| | 131091.1612 | 2.5 L |
| | 131091.1214 | 5 L |
| | 131091.0716 | 25 L |
| **Purified Water (BP, Ph. Eur.) pure, pharma grade** | 141074.1315 | 10 L |

## Reagents for the preparation of buffer solutions

| Product name | Code | Package |
|---|---|---|
| **Potassium di-Hydrogen Phosphate (USP-NF, BP, Ph. Eur.) pure, pharma grade** | 141509.1210 | 500 g |
| | 141509.1211 | 1000 g |
| | 141509.1214 | 5 kg |
| **di-Potassium Hydrogen Phosphate anhydrous (BP, Ph. Eur.) pure, pharma grade** | 141512.1211 | 1000 g |
| **di-Sodium Hydrogen Phosphate 12-hydrate (USP, BP, Ph. Eur.) pure, pharma grade** | 141678.1211 | 1000 g |
| | 141678.1214 | 5 kg |
| **Sodium Hydroxide 0.2 mol/l (0.2N) standard volumetric solution** | 182971.1211 | 1000 ml |
| **Sodium Hydroxide 1 mol/l (1N) volumetric solution** | 181691.1211 | 1000 ml |
| | 181691.1212 | 2.5 L |
| | 181691.1214 | 5 L |
| | 181691.1315 | 10 L |

## Reagents for the preparation of culture media

| Product name | Code | Package |
|---|---|---|
| **Agar, Bacteriological European Type (Ingredient) for microbiology** | 402302.1210 | 500 g |
| | 402302.0914 | 5 kg |
| **Agar Bacteriology grade** | A0949,0500 | 500 g |
| | A0949,1000 | 1 kg |
| | A0949,5000 | 5 kg |
| **D(+)-Glucose 1-hydrate (USP, BP, Ph. Eur.) pure, pharma grade** | 143140.1211 | 1 kg |
| **Meat Extract (Ingredient) for microbiology** | 403692.1210 | 500 g |
| **Peptone, Bacteriological (Ingredient) for microbiology** | 403695.1210 | 500 g |
| **Peptone from Casein (Ingredient) for microbiology** | 403898.1210 | 500 g |
| **Peptone from Casein (acid hydrolysate)** | A2142,0500 | 500 g |
| **Peptone from Casein (enzymatic digest)** | A2208,0500 | 500 g |
| **Peptone from Casein (pancreatic digest)** | A2210,0500 | 500 g |
| **Peptone from Soybean (enzymatic digest)** | A2206,1000 | 1 kg |
| **Peptone from Meat (enzymatic digest)** | A1835,0500 | 500 g |
| **Potassium di-Hydrogen Phosphate (USP-NF, BP, Ph. Eur.) pure, pharma grade** | 141509.1210 | 500 g |
| | 141509.1211 | 1 kg |
| | 141509.1214 | 5 kg |
| **di-Potassium Hydrogen Phosphate anhydrous (BP, Ph. Eur.) pure, pharma grade** | 141512.1211 | 1 kg |
| **Potassium Nitrate without anticaking (Reag. Ph. Eur.) for analysis, ISO** | 131524.1210 | 500 g |
| | 131524.1211 | 1 kg |
| | 131524.1214 | 5 kg |



>>

<<

| Product name | Code | Package |
|---|---|---|
| **Sodium Chloride (USP, BP, Ph. Eur., JP) pure, pharma grade** | 141659.1210 | 500 g |
| | 141659.1211 | 1 kg |
| | 141659.1214 | 5 kg |
| **di-Sodium Hydrogen Phosphate 12-hydrate (USP, BP, Ph. Eur.) pure, pharma grade** | 141678.1211 | 1 kg |
| | 141678.1214 | 5 kg |
| **Tween® 80 BioChemica** | A1390,0500 | 500 g |
| | A1390,1000 | 1 kg |
| **Tween® 80 (USP-NF, BP, Ph. Eur.) pure, pharma grade** | 142050.1611 | 1000 ml |
| | 142050.1214 | 5 L |
| **Yeast Extract (Ingredient) for microbiology** | 403687.1210 | 500 g |
| **Yeast Extract BioChemica** | A1552,0500 | 500 g |
| | A1552,1000 | 1 kg |


## Antibiotics

PanReac AppliChem does not provide the standards but a large number of antibiotics that can be used in cell culture and other experimental assays.

| Product name | Code |
|---|---|
| **Actinomycin D BioChemica** | A1489 |
| **Amikacin Sulfate BioChemica** | A8694 |
| **Amphotericin B BioChemica** | A1907 |
| **Amphotericin B (Ph. Eur.) pure, pharma grade** | A7009 |
| **Ampicillin Sodium Salt BioChemica** | A0839 |
| **Ampicillin Sodium Salt (Ph. Eur.) pure, pharma grade** | A6352 |
| **Apramycin Sulfate BioChemica** | A7682 |
| **Bacitracin BioChemica** | A0623 |
| **Blasticidin S Hydrochloride BioChemica** | A3784 |
| **Carbenicillin Disodium Salt BioChemica** | A1491 |
| **CellCultureGuard** | A8906 |
| **Chloramphenicol BioChemica** | A1806 |
| **Chloramphenicol pure Ph. Eur.** | A6435 |
| **Colistin Sulfate BioChemica** | A2922 |
| **Cycloheximide BioChemica** | A0879 |
| **D-Cycloserine BioChemica** | A1943 |
| **Cytochalasin B BioChemica** | A7657 |
| **Doxycycline Hyclate BioChemica** | A2951 |
| **G418 Disulfate BioChemica** | A2167 |
| **G418 Disulfate solution, sterile** | A6798 |
| **Gentamycin Sulfate BioChemica** | A1492 |
| **Gentamycin Sulfate (Ph. Eur.) pure, pharma grade** | A4854 |

>>

<<

| Product name | Code |
|---|---|
| **Hygromycin B** | A5347 |
| **Hygromycin B solution** | A2175 |
| **Kanamycin Sulfate BioChemica** | A1493 |
| **Kanamycin Sulfate (Ph. Eur., BP) pure, pharma grade** | A4789 |
| **Mitomycin C** | A2190 |
| **Mupirocin (USP) pure, pharma grade** | A4718 |
| **N6-2'-O-Dibutyryl-Adenosine 3',5'-Cyclophosphate Sodium Salt 1-hydrate** | A0455 |
| **Nalidixic acid BioChemica** | A1894 |
| **Nigericin sodium salt BioChemica** | A7671 |
| **Novobiocin Sodium Salt BioChemica** | A1944 |
| **Nystatin 2-hydrate BioChemica** | A3811 |
| **Oxytetracycline Hydrochloride** | A5257 |
| **Penicillin G Potassium Salt BioChemica** | A1837 |
| **Penicillin - Streptomycin (100X) for cell culture** | A8943 |
| **Polymyxin B Sulfate BioChemica** | A0890 |
| **Puromycin Dihydrochloride BioChemica** | A2856 |
| **Rifampicin** | A2220 |
| **Spectinomycin Dihydrochloride 5-hydrate BioChemica** | A3834 |
| **Staurosporine BioChemica** | A7626 |
| **Tetracycline Hydrochloride** | A2228 |
| **Tunicamycin** | A2242 |
| **Vancomycin hydrochloride BioChemica** | A1839 |

# Package pictograms

| | |
|---|---|
|  | Glass bottle |
|  | Plastic bottle |
|  | Plastic jerrycan |
|  | Plastic bucket |
|  | Sol-Pack: Plastic container in a carton box (cubitainer), with tap |
|  | Paperboard box |
|  | Plastic spray bottle |
|  | Plastic tube |

A195-3,EN;201807

PanReac AppliChem
ITW Reagents

**AppliChem GmbH**
Ottoweg 4 · DE-64291 Darmstadt · Germany · Phone +49 6151 9357 0 · Fax +49 6151 9357 11
**info.de@itwreagents.com**

**Nova Chimica Srl**
Via G. Galilei, 47 · I-20092 Cinisello Balsamo · (Milano) Italy · Phone +39 02 66045392 · Fax +39 02 66045394
**info.it@itwreagents.com**

**PanReac Química SLU**
C/ Garraf 2, Polígono Pla de la Bruguera · E-08211 Castellar del Vallès · (Barcelona) Spain · Phone +34 937 489 400 · Fax +34 937 489 401
**info.es@itwreagents.com**

**www.itwreagents.com**