DNA Isolation Spin-Kit Agarose

Kit for the DNA isolation from agarose gels with spin minicolumns
Product No. A5193

Description

Introduction

The DNA Isolation Spin-Kit Agarose is based on the ability of DNA to adsorb to silica surfaces in the presence of high concentrations of chaotropic salts. The agarose gel slice containing the DNA of interest is dissolved in **Agarose Melting Solution AC** containing chaotropic salts. In the next step, the **isopropanol** is added and the whole sample is loaded onto a minicolumn with a suitable silica resin. The DNA binds to the silica resin, while impurities freely pass through the column. Residual impurities are washed off. Pure DNA is eluted with a low salt buffer or water. The DNA can be used in cloning or sequencing experiments without further purification.

Kit Components sufficient for 50 isolations

Order-No.:	A5193,0050A	Agarose Melting Solution AC	30	ml
Order-No.:	A5193,0050B	Isopropanol	15	ml
Order-No.:	A5193,0050C	Wash Solution AC1	50	ml
Order-No.:	A5193,0050D	MiniColumns ACAG	50	pcs
Order-No.:	A5193,0050E	Sodium acetate (3M, pH 5,5)	1	ml
Order-No:	A5193,0050F	TE buffer	5	ml

Storage: Room Temperature
Stability: min. 12 months

Features

Applications:	cloning, sequencing
Advantages:	-70 % DNA recovery, fast protocol
Material:	silica membrane
Binding capacity:	10 µg
Operate:	spin, vacuum
Elution solution:	TE buffer, Tris buffer, water
elution volume:	30 - 50 µl

Notes

- The agarose gel electrophoresis can be performed in the presence of either TAE or TBE buffer.
- The DNA Isolation Spin-Kit allows the purification of DNA fragments in the range of 100 to 10.000 bp.
- The binding capacity of the Spin-Kit minicolumn resin is up to 10 µg DNA.

Equipment and materials necessary for DNA purification that are not included in kit

1. Agarose slice containing the DNA sample
2. Sterile water (nuclease free, DEPC treated
3. Sterile 1.5 ml Eppendorf tubes
4. Benchtop microcentrifuge
5. Heatblock or incubator capable of working at 50 °C

NOTE:
Before you start working, we recommend cleaning the work surface using
DNA-Exitus Plus or DNA-Exitus Plus IF (A7089 & A7409)

Protocol

1. Cut out the agarose slice containing the DNA of interest.
   Attention! The weight of the agarose slice should not exceed 200 mg.
2. Transfer the agarose slice to a 1.5 ml microcentrifuge tube (not supplied) and add **Agarose Melting Solution AC:**
   < 2% agarose gel - 400 µl
   ≥ 2% agarose gel - 500 µl
3. Incubate the sample at 50°C until the agarose is dissolved completely. Mix from time to time by inverting the tube or by vortexing.
   Agarose melting solution R7SI contains the colour pH indicator. Upon mixing the DNA sample with R7SI agarose melting solution. Yellow colour of the mixture indicates an optimal pH for DNA binding.
   If the mixture colour turns pink the pH of the solution is too high. In such conditions DNA binds inefficiently to the silica membranes and may be lost.
   If the colour of the solution is pink adjust the pH by adding 1-10 µl of 3M Sodium acetate solution (pH 5.5) (included) and mix. As soon as the colour of the mixture turns yellow, proceed with the purification protocol.
4. Add an appropriate volume of Isopropanol:
   < 2% agarose gel - 200 µl
   ≥ 2% agarose gel - 250 µl
   Mix by inverting the tubes.
5. Centrifuge briefly the samples to remove the leftovers of solution from the tube walls and caps.
6. Apply the whole mixture on the minicolumns
7. Spin for 30 seconds at 10 000 - 15 000 rpm.
8. Remove the MiniColumn ACAG from the tube, discard flow-through and attach the MiniColumn ACAG to the same tube.
9. Add 600 µl of **Wash Solution AC1**.
10. Spin tor 30 seconds at 10 000 -15 000 rpm.
11. Remove the MiniColumn ACAG from the tube, discard flow-through and attach the MiniColumn ACAG to the same tube.
12. Add 300 µl of **Wash Solution AC1**.
13. Spin for 2 minute at 10 000 - 15 000 rpm.
14. Place the dry MiniColumn ACAG into a new 1.5 ml reaction tube (not provided) and add 30-50 µl of TE buffer or sterile water directly onto the minicolumns resin.
    Attention! While eluting the DNA with TE buffer or water, be sure that the liquid is applied precisely onto the resin. If liquid remains on the column walls, the elution may not be effective.
15. Incubate for 3 minutes at room temperature.
16. Spin for 1 minute at 10 000 - 15 000 rpm.
17. Store the reaction tube containing the pure DNA at +4°C.