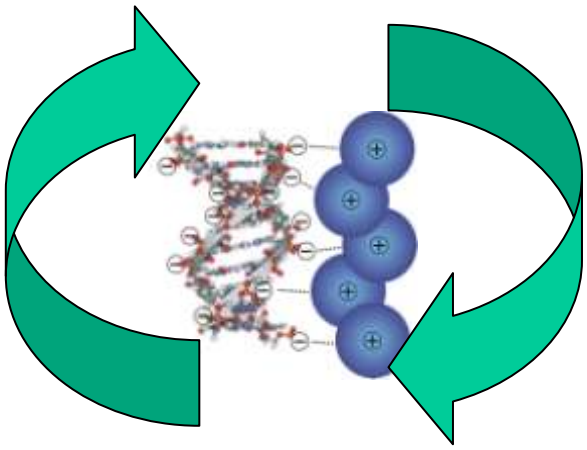
PanReac AppliChem
ITW Reagents

# MAXXBOND

*ready-to-use - Kit for the regeneration of DNA binding columns with pure silica matrices*
**Product No. MB007**

## Nucleic acid-free silica-matrix: Regeneration of DNA binding columns



MAXXBOND

- efficient and easy handling, only two washing steps
- complete removal of all nucleic acids (both free and trapped)
- no damage to the silica matrix
- full binding capacity after regeneration
- successfully tested for all methods in molecular biology and gene technology (e. g. sequencing, cloning, transformation, ...)
- environmentally friendly: All components of the **MAXXBOND** kit are bio-degradable and non-toxic for humans.
- drastic reduction of mini column waste
- cost saving

**Attention:** Don't discard used plastic materials from DNA isolation kits!

**Kit components:**

| **Product number** | **MB007,0100** | **MB007,0200** | **MB007,1000** |
|---|---|---|---|
| RG1 (Removal of residual nucleic acids) | MB007,0100RG1 100ml | MB007,0200RG1 200ml | MB007,1000RG1 1L |
| RG2 (Regeneration of binding capacity) | MB007,0100RG2 100 ml | MB007,0200RG2 200ml | MB007,1000RG2 1L |
| Product information | ✓ | ✓ | ✓ |

**Get it all from us** AppliChem supplies the Plas/mini Isolation kit with spin columns (product number A5172,0050 and A5172,0250 with 50 and 250 mini columns, respectively), the regeneration system MAXXBOND for silica-based columns (MB007) and the refill buffer set MAXXMORE (MB008). All three components are optimized for use in combination, but may be as well mixed with components (buffers, columns) of other suppliers.

**Activity:** Regeneration solution 1 destroys approximately 1 µg of cccDNA within 2 minutes.

**Storage:** Room temperature
Storage below 20°C may cause the formation of a precipitate. Completely dissolves after warming to 40 - 50°C.

**Stability:** RG1 is stable for 20 months from the date of production.
The reagent contains a colour indicator. The solution is clear and colourless or light yellow, but turns to dark brown during storage. It is sensitive to oxygen. Product is filled under argon! When the color of RG1 turns to dark brown after 12 months, its activity is not reduced.
RG2 is stable for several years.

**Colour indicator of RG1**

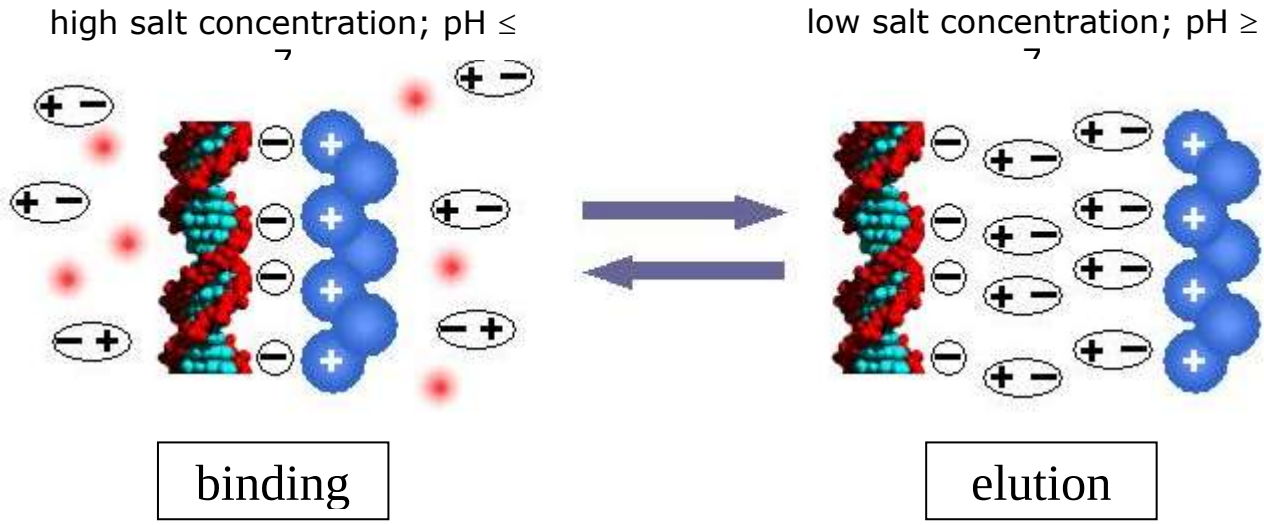
| 0 months | 3 months | 6 months | 9 months | 12 months |
|---|---|---|---|---|

**Safety:** Solutions don't contain hazardous substances.
For research and laboratory use only.

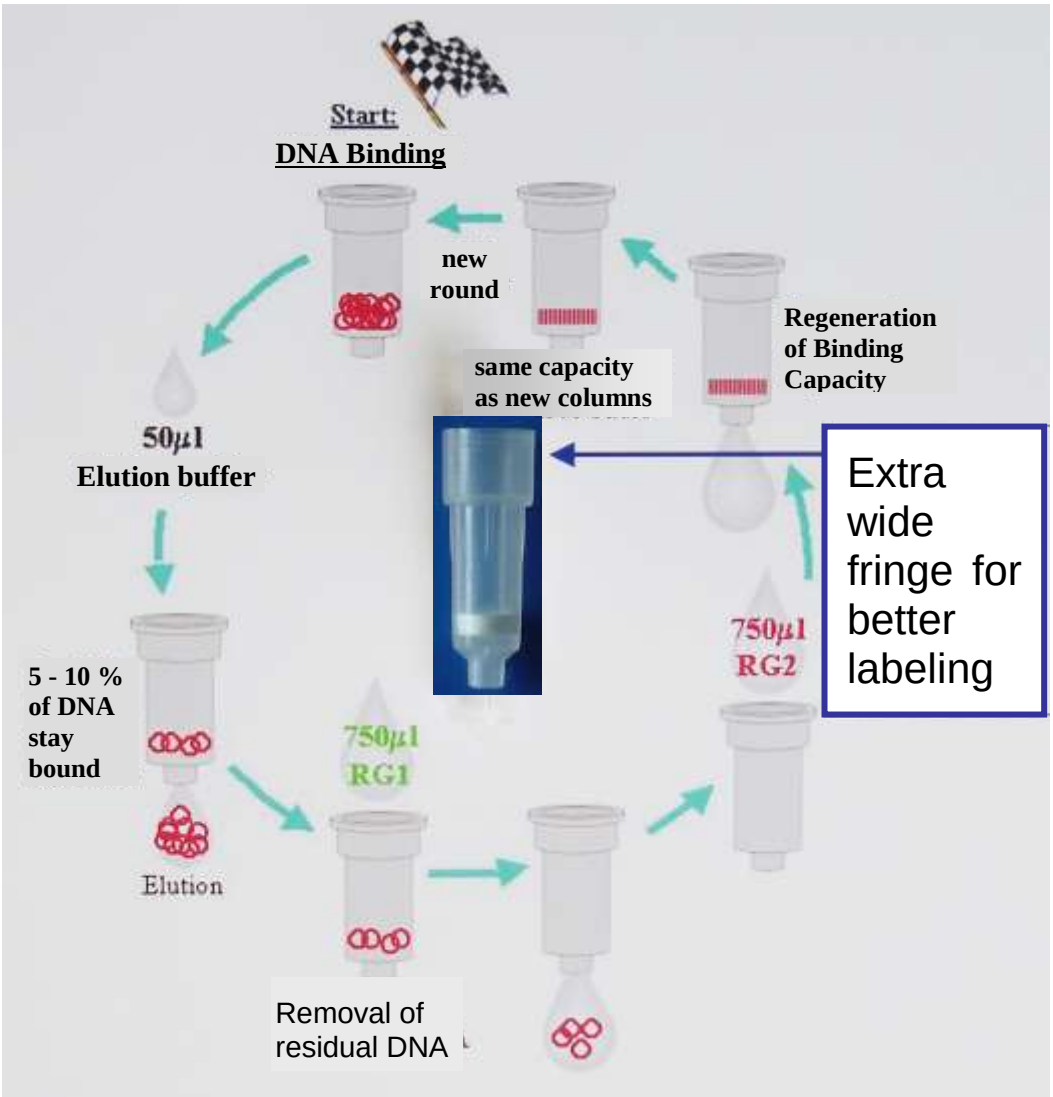
**Effects on materials, equipment and the skin:**
No corrosive effects of either RG1 or RG2 on metal or plastic surfaces have been observed.
RG1 is slightly irritating to skin. In case of contact, wash with plenty of water.

**Principle of Purification of Silica Matrices**

The purification principle is based on the negative charge of the phosphate residues of the DNA molecules and the positive charged surface of the silica. This principle is identical for all pure silica matrices.




**Regeneration Cycle**



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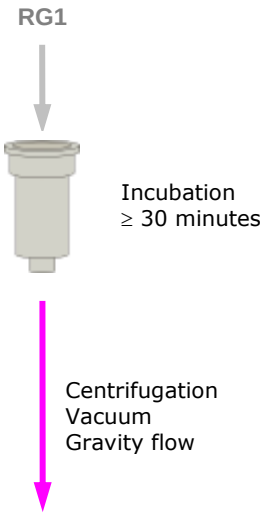
# Protocol 1

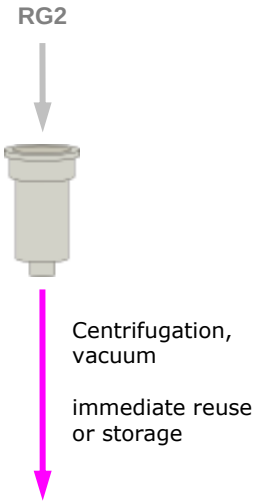
**Fast regeneration (approximately 30 minutes) of mini columns for multiple binding of CCC-form plasmid DNA**

**1.** After elution of the plasmid DNA sample **apply <u>750 µl of RG1</u>.**
Incubate for a minimum of 30 minutes. Shorter incubations times may be sufficient for weakly contaminated columns (10 minutes). Longer incubation times won't damage the column material, but may improve the result. If the solution passes through large columns too fast, seal the top with Parafilm® (American National Can Company, USA). Mini columns won't run dry, since capillary forces will keep enough solution in the column.
After incubation, let the 750 µl solution of RG1 pass through the column completely (use centrifugation, vacuum or gravity flow).




**Heavily contaminated columns**
For columns with high levels of DNA contaminations, 10 minutes incubation with RG1 may not be sufficient to eliminate any residual DNA. Therefore, increase the incubation time (30 minutes to 24 hours). Detailed tests have shown that incubation of 1 hour was sufficient to eliminate any DNA from heavily contaminated columns or from columns that have been stored for a longer period of time before regeneration.

**2.** In a second step **apply <u>750 µl of RG2</u>**. Let the solution pass through the column completely. Use centrifugation, vacuum or gravity flow.




With **MAXXBOND** several column sizes may be regenerated. Adjust the volume of RG1 and RG2 used according to the table:

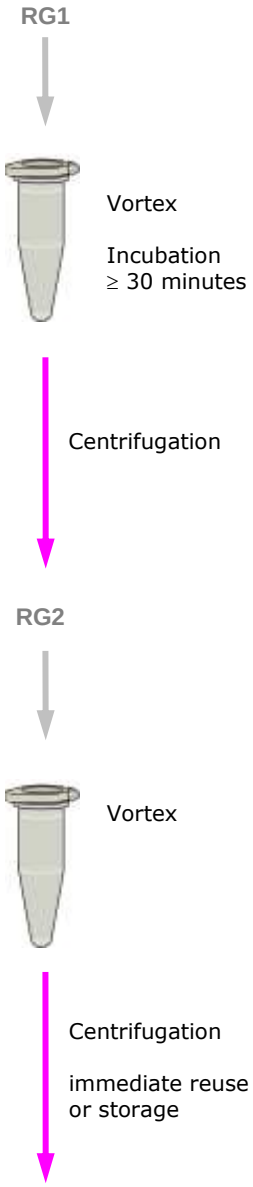
| Column type | RG1 & RG2 |
|---|---|
| Mini-Column | 750 µl each |
| 96 well plates | 750 µl/well |

**3.** Immediately after the last washing step with RG2 the mini column can be reused for binding of plasmid DNA.
Alternatively, the column can be stored at room temperature until the next binding procedure.

**No damage to the DNA binding matrix:**
Columns were incubated for 5 minutes to 24 hours with RG1 and RG2 without causing any damage to the silica matrices and without loss of binding capacity - even after reuse up to 20 times.

# Protocol 2

**Fast regeneration of silica particles / glass powder (glass milk) for multiple binding of DNA**




**1.** After elution of the DNA sample **apply <u>500 µl of</u>** RG1 per 10 µl or silica particles or glass powder. Incubate for at least 30 minutes.
Longer incubation times won't damage the silica material, but may improve the result.

**Larger quantities of silica particles / glass powder**
For the daily work in the laboratory, it is more efficient to collect and regenerate larger quantities of the silica particles. Up to 50 µl of silica particles / glass powder may be regenerated in 500 µl of solution RG1.
As long as a 10 times larger volume of the solutions RG1 and RG2 in relation to the silica particles / glass powder is applied, the removal of any residual DNA is assured.

**2.** In a second step **apply <u>500 µl of RG2</u>** to the silica particles / glass powder and vortex. Remove the RG2 solution after centrifugation.

**1.) DNA purity**

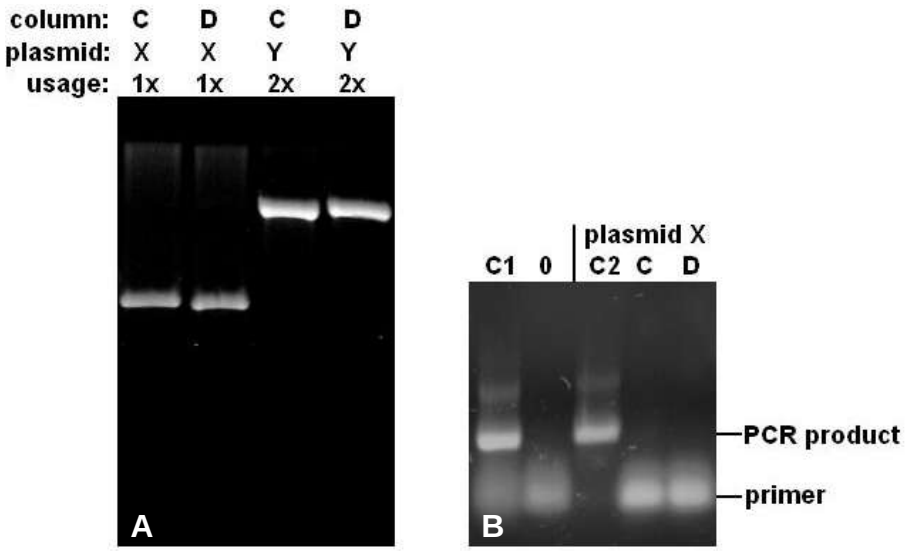



**Fig. 1 A + B**
**Demonstration that MAXXBOND -regenerated columns do not contain residual DNA molecules from the former isolation**. The two columns C and D were used for the isolation of plasmid X. After regeneration of the columns a second plasmid Y was purified with the identical columns. The analytical agarose gel (**A**) shows identical aliquots of the DNA isolations. The very different molecular weights of X and Y allow the rapid identification of any contamination.
The DNA isolation of sample Y (A) do not contain any traces of the first DNA sample X. Plasmid DNA was linearised by restriction enzyme digestion before gel electrophoresis. The PCR test (**B**) does not detect any DNA molecules from the first isolation. Before the purification of DNA sample Y, column C was treated for 24 hours and column D for 5 minutes with RG1. Then, 750 µl RG2 was applied to each column. Finally, 50 µl of elution buffer (10 mM Tris, pH 8.0) were centrifuged through the columns. From these 50 µl eluates aliquots of 2 µl were added to 50 µl PCR reaction mixtures together with the appropriate primers for insert X. (C1: Control with plasmid X DNA (1 ng); 0: no template X DNA; C2: Control with plasmid X DNA (1 ng) und 2 µl of each eluate after the regeneration of column C and D; C: 2 µl eluate from column C after isolation of plasmid X and regeneration (24 h); D: 2 µl eluate from column D after isolation of plasmid X and regeneration (5 min.).

**2.) Binding capacity**

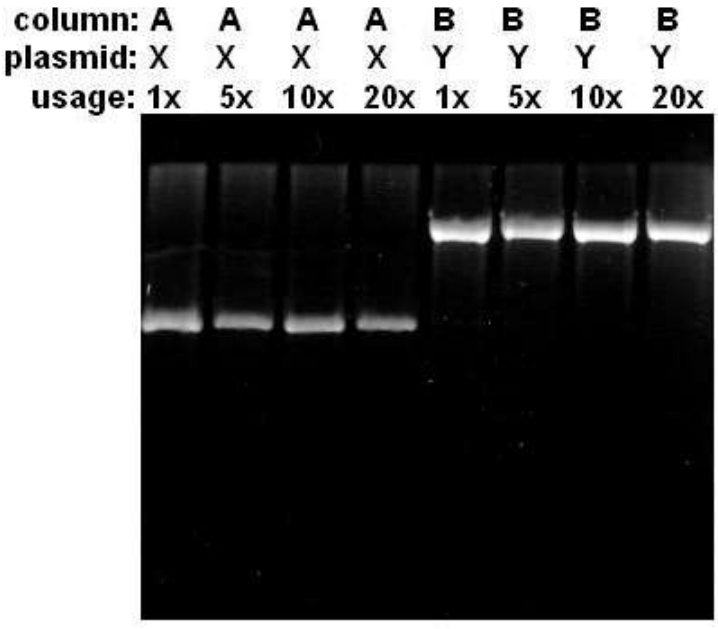



**Fig. 2**
**Demonstration of the identical binding capacity of DNA binding columns after 20 uses and regeneration with MAXXBOND**. The two columns A and B were used for 20 cycles of isolation and regeneration of plasmid DNA from identical aliquots of recombinant *E. coli* cultures. Identical aliquots of the eluted DNA samples were analysed on an agarose gel. After staining with ethidium bromide the gel was photographed. Plasmid DNA was linearised by restriction enzyme digestion before gel electrophoresis.

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### 3.) DNA Sequencing

Four independent DNA preparations of the same plasmid, isolated with columns regenerated several times with **MAXXBOND**, have been sequenced by the independent sequencing laboratory Sequiserve GmbH. As a control, one sample (REG-4) has been additionally purified by Sequiserve with their own column system, providing optimal conditions for the sequencing.
All four clones gave brilliant results in terms of readability of the sequences up to 750 bp. Not a single nucleotide differed between all four samples. Electropherograms could even be analyzed up to approximately 1000 bp. REG-1 and REG-2 are AppliChem columns, while REG-3 is a column of another supplier. All columns have been reused at least 5 times before preparations for sequencing. All DNA isolations have been performed with buffer solutions from AppliChem (washing buffers, lysis buffer, elution buffer, ..., S1 - S5).

>REG-1 / 5 min. RG1+ / AppliChem / (M13rev)
ATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGTATTGAATTTGAAGCATGAAATCGTGCTTATCAATTTTAT
GTCACCCTAAAACATCTGTACGTGTTTATATAGATATTTAAAGCAATATTTGCCAGGATTTGGTGAAGATCCCTCATATAACTCTCATAAATG
CGGATTTTCGGAGCGAAAAAAGCCTAAATTCTTGTCTGGAAGTATAATTGGCGGTGAAATAGAAAAGGTGGCAATCACGACTGAAAAGGGTAC
AGCTTTCGCAACTGACATATACAGACGGTGAAAAGTAATAAATTGCCCAAGTGTGAACATGTCAGGTGTAAGCTCTGTTATGCTCGGTCTTCG
ACCTGCTACAAGAATTTTTTTCCGCAGTAATATTTCGGTTTCACCTTCGAGGACTTTTGTATCATATATTGGAAGATCCCAGAGCACGTCGAT
ACTCAAAAATGCTCCCAACTTAGAGGACAATGTCACAAATCTTCAGAAAATTATACCGAAACGGTTCTTTTCTCAAACATCAATTTTGAAATC
AAGGTGGAAGCCTATATTCAATGAAGAAACTACTAATCGATACGTACGTTTGAACAGGTTTCAGCAGTACCAGCAGCAGAGAAGCGGCGGCAA
TCCTCTGGGCTCTATGACTATTTTGGGGCTCTCTTTAATGGCAGGAATATATTTTGGCTCCCCTTATTTGTTCGAGCACGTTCCACCCTTTAC
GTATTTTAAGACGCATCCAAAGAATCTGGTATACGCGTTA

>REG-2 /5 min. RG1+ / AppliChem / (M13rev)
ATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGTATTGAATTTGAAGCATGAAATCGTGCTTATCAATTTTAT
GTCACCCTAAAACATCTGTACGTGTTTATATAGATATTTAAAGCAATATTTGCCAGGATTTGGTGAAGATCCCTCATATAACTCTCATAAATG
CGGATTTTCGGAGCGAAAAAAGCCTAAATTCTTGTCTGGAAGTATAATTGGCGGTGAAATAGAAAAGGTGGCAATCACGACTGAAAAGGGTAC
AGCTTTCGCAACTGACATATACAGACGGTGAAAAGTAATAAATTGCCCAAGTGTGAACATGTCAGGTGTAAGCTCTGTTATGCTCGGTCTTCG
ACCTGCTACAAGAATTTTTTTCCGCAGTAATATTTCGGTTTCACCTTCGAGGACTTTTGTATCATATATTGGAAGATCCCAGAGCACGTCGAT
ACTCAAAAATGCTCCCAACTTAGAGGACAATGTCACAAATCTTCAGAAAATTATACCGAAACGGTTCTTTTCTCAAACATCAATTTTGAAATC
AAGGTGGAAGCCTATATTCAATGAAGAAACTACTAATCGATACGTACGTTTGAACAGGTTTCAGCAGTACCAGCAGCAGAGAAGCGGCGGCAA
TCCTCTGGGCTCTATGACTATTTTGGGGCTCTCTTTAATGGCAGGAATATATTTTGGCTCCCCTTATTTGTTCGAGCACGTTCCACCCTTTAC
GTATTTTAAGACGCATCCAAAGAATCTGGTATACGCGTTA

>REG-3 /5 min. RG1+ / Market Leader / (M13rev)
ATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGTATTGAATTTGAAGCATGAAATCGTGCTTATCAATTTTAT
GTCACCCTAAAACATCTGTACGTGTTTATATAGATATTTAAAGCAATATTTGCCAGGATTTGGTGAAGATCCCTCATATAACTCTCATAAATG
CGGATTTTCGGAGCGAAAAAAGCCTAAATTCTTGTCTGGAAGTATAATTGGCGGTGAAATAGAAAAGGTGGCAATCACGACTGAAAAGGGTAC
AGCTTTCGCAACTGACATATACAGACGGTGAAAAGTAATAAATTGCCCAAGTGTGAACATGTCAGGTGTAAGCTCTGTTATGCTCGGTCTTCG
ACCTGCTACAAGAATTTTTTTCCGCAGTAATATTTCGGTTTCACCTTCGAGGACTTTTGTATCATATATTGGAAGATCCCAGAGCACGTCGAT
ACTCAAAAATGCTCCCAACTTAGAGGACAATGTCACAAATCTTCAGAAAATTATACCGAAACGGTTCTTTTCTCAAACATCAATTTTGAAATC
AAGGTGGAAGCCTATATTCAATGAAGAAACTACTAATCGATACGTACGTTTGAACAGGTTTCAGCAGTACCAGCAGCAGAGAAGCGGCGGCAA
TCCTCTGGGCTCTATGACTATTTTGGGGCTCTCTTTAATGGCAGGAATATATTTTGGCTCCCCTTATTTGTTCGAGCACGTTCCACCCTTTAC
GTATTTTAAGACGCATCCAAAGAATCTGGTATACGCGTTAT

Control:
>REG-4 / 5 min. RG1+ / AppliChem / +Sequiserve Column (M13rev)
ATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGTATTGAATTTGAAGCATGAAATCGTGCTTATCAATTTTAT
GTCACCCTAAAACATCTGTACGTGTTTATATAGATATTTAAAGCAATATTTGCCAGGATTTGGTGAAGATCCCTCATATAACTCTCATAAATG
CGGATTTTCGGAGCGAAAAAAGCCTAAATTCTTGTCTGGAAGTATAATTGGCGGTGAAATAGAAAAGGTGGCAATCACGACTGAAAAGGGTAC
AGCTTTCGCAACTGACATATACAGACGGTGAAAAGTAATAAATTGCCCAAGTGTGAACATGTCAGGTGTAAGCTCTGTTATGCTCGGTCTTCG
ACCTGCTACAAGAATTTTTTTCCGCAGTAATATTTCGGTTTCACCTTCGAGGACTTTTGTATCATATATTGGAAGATCCCAGAGCACGTCGAT
ACTCAAAAATGCTCCCAACTTAGAGGACAATGTCACAAATCTTCAGAAAATTATACCGAAACGGTTCTTTTCTCAAACATCAATTTTGAAATC
AAGGTGGAAGCCTATATTCAATGAAGAAACTACTAATCGATACGTACGTTTGAACAGGTTTCAGCAGTACCAGCAGCAGAGAAGCGGCGGCAA
TCCTCTGGGCTCTATGACTATTTTGGGGCTCTCTTTAATGGCAGGAATATATTTTGGCTCCCCTTATTTGTTCGAGCACGTTCCACCCTTTAC
GTATTTTAAGACGCATCCAAAGAATCTGGTATACGCGTTATT

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www.sequiserve.de

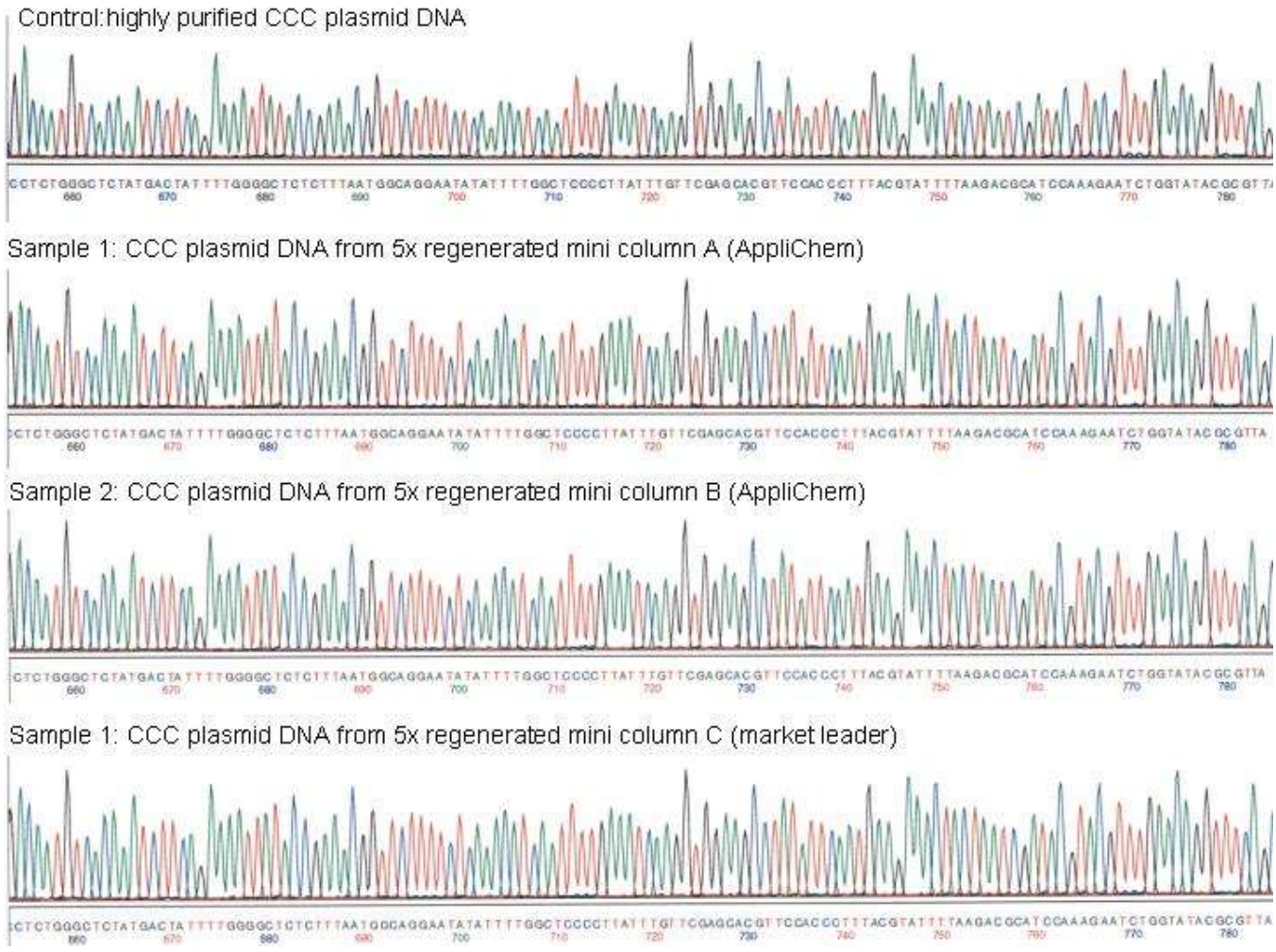
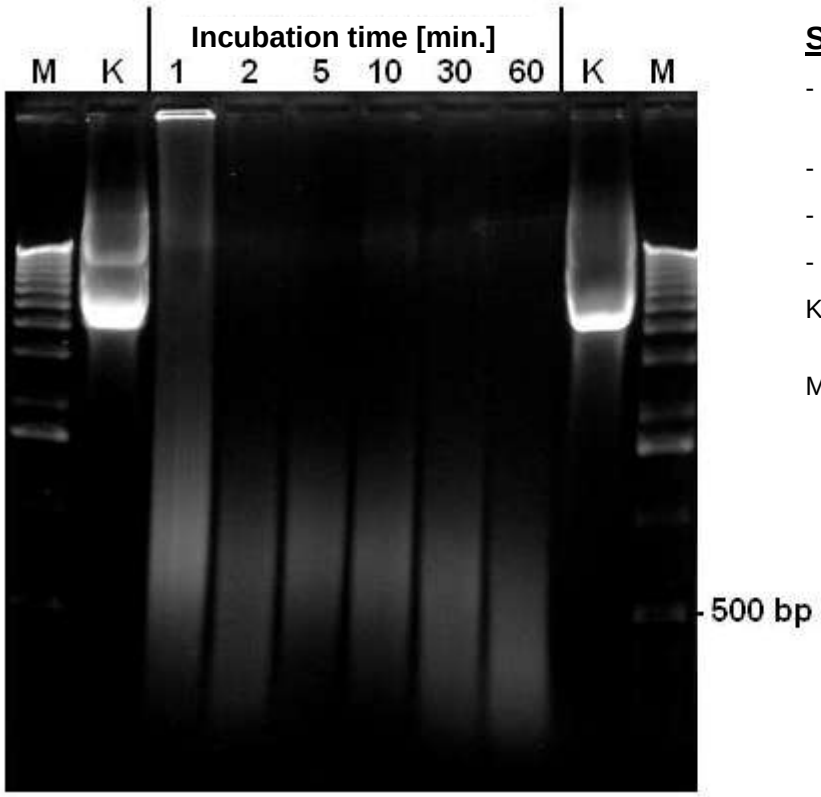



Fig. 3

**Sequencing of DNA samples, purified with MAXXBOND-regenerated DNA binding columns**. Sequences can be analysed up to approximately 1000 bp. Not a single nucleotide exchange could be observed or any other difference caused by contamination.

**4.) Time-dependence of DNA destruction by MAXXBOND RG1**

To analyze the time-dependence of the DNA destruction by RG1, 1 µg of ccc-plasmid DNA was incubated for 1, 2, 5, 10, 30, and 60 minutes, respectively, with only 5 µl of the MAXXBOND component RG1 in micro-reaction tubes.
Figure 4 shows that after 5 minutes of incubation not all DNA is completely destroyed. In this case PCR-fragments might be generated, but no contaminations by intact plasmids. The destruction by MAXXBOND is extremely fast: Larger fragments are destroyed immediately, resulting in no replicable vectors after 5 minutes. Smaller fragments, detectable by PCR, will sometimes be present even after incubation for up to 10 minutes. Therefore, our control tests have been performed with primers resulting in very small PCR fragments. After longer incubation times, the small fragments will be destroyed completely. Linearized DNA molecules will be destroyed faster than ccc-plasmid DNA (Data not shown).




**Sample preparation**:

- 1 µg ccc-plasmid DNA in 5 µl $H_2O$ + 5 µl RG1 MAXXBOND
- Incubation [min.] at room temperature
- Denaturation for 5 min. at 97°C
- loading of 1 µg DNA sample per lane

K control 1 µg ccc-plasmid DNA in 5 µl $H_2O$ + 5 µl $H_2O$

M Marker 1 kb ladder

**Fig. 4**
**Time-dependence of DNA destruction by MAXXBOND RG1**. After 2 minutes of incubation, no replicable plasmids or larger DNA fragments are detectable.

**5.) Stability of DNA isolated with MAXXBOND-regenerated columns**

DNA, isolated with AppliChem's refill buffer solutions (lysis buffer, washing buffers, elution buffer etc.) and regenerated columns, were incubated digested or undigested for up to 96 hours at 36°C. No degradation of the DNA could be observed. This shows that RG1 will not affect the new DNA after regeneration of the columns.

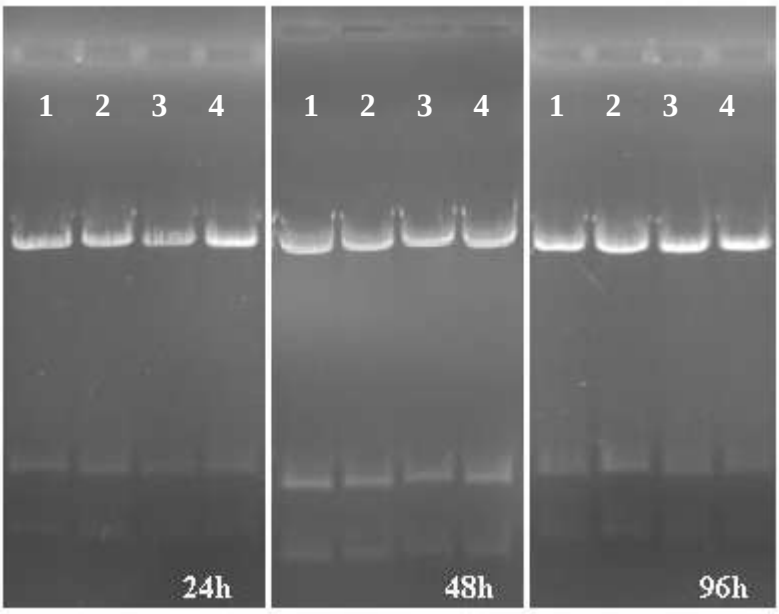



Fig. 5

**Stability of DNA isolated with MAXXBOND-regenerated columns.** Four independent plasmid isolations using regenerated columns and AppliChem's refill buffer solutions, have been linearized at 36°C with the restriction enzyme *Eco* RI for the intervals given.

**6.) Regeneration of DNA binding columns for purification of PCR fragments**

Besides the regeneration of the classical DNA binding columns for the purification of plasmid DNA, we can show the regeneration of the columns for the purification of PCR fragments. A treatment of 10 minutes with MAXXBOND is sufficient to remove all contaminating DNA.
So far, PCR fragments of 3 kb to 500 bp have been isolated with regenerated columns without any problems. Since MAXXBOND does not alter the properties of the silica matrix, the properties of the purification columns won't change too. The purification of very small PCR fragments depends on the composition and the ion strength of the buffer. It is independent of the source (e. g. single cells, templates, mixtures). If the corresponding buffers are used, regenerated columns will behave like new columns.

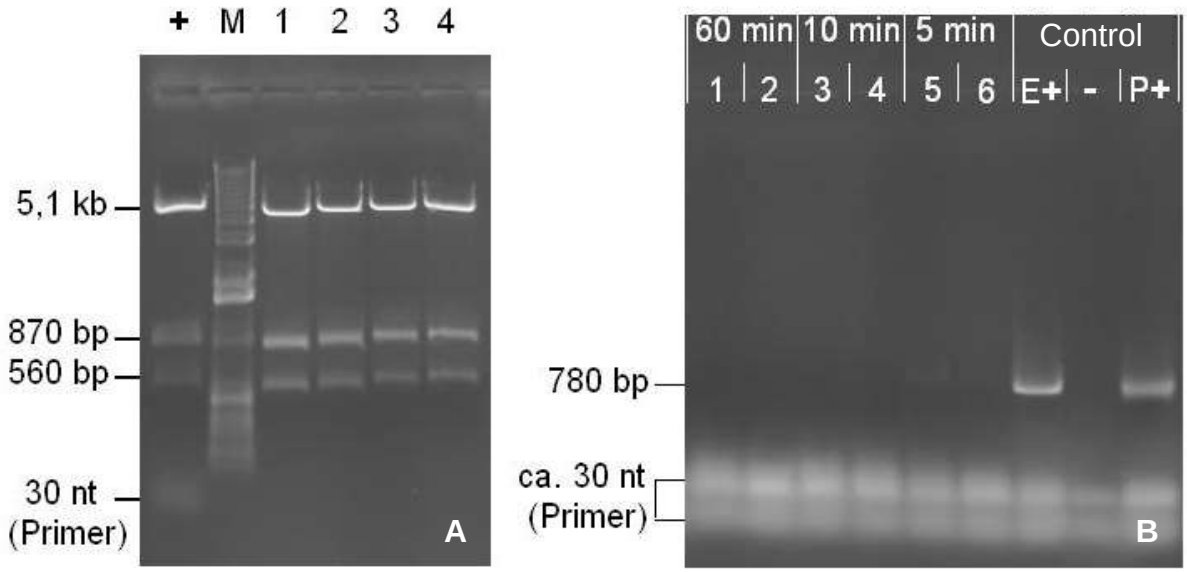



**Fig. 6**
**Regeneration of DNA binding columns for the purification of PCR fragments. Recovery of the full DNA binding capacity (A) and proof of the removal of all nucleic acids (B)**. Test for the binding capacity of 10x regenerated DNA purification columns of PCR fragments with commercial standard buffer and newly formulated MAXXMORE PCR fragment buffers. A mixture of linear DNA fragments was isolated with 10x regenerated columns and eluted each with 50 µl of 10 mM Tris (pH 8.0), respectively. 8 µl of each eluate were analysed on a 0.8 % agarose gel. Lanes: +: Mix of 3 DNA fragments (ca. 500 ng) before the purification, M: molecular weight marker, 1 and 2: Purification with columns and buffers of the market leader, 3 and 4: purification with newly formulated MAXXMORE PCR fragment buffers.

Part B of the figure shows the PCR test for the complete removal of DNA fragments after regeneration with MAXXBOND. After 10 cycles of isolation and regeneration, the columns have been used for the purification of linear test DNA fragments, and subsequently regenerated with MAXXBOND. Elution has been performed with 50 µl of 10 mM Tris (pH 8.0), and 2 µl each of the eluate have been analysed by PCR for the complete removal of DNA. 10 µl of the PCR reaction were analysed on a 0.8 % agarose gel. Lanes: 1 and 2: 60 min. regeneration, 3 and 4: 10 min. regeneration, 5 and 6: 5 min. regeneration, E+: PCR with 2 µl eluate from regenerated columns + 50 ng template, -: negative control (PCR without template), P+: positive control (PCR with 50 ng template). A treatment of 10 minutes with MAXXBOND is sufficient to remove all contaminating DNA.

# Trouble Shooting Guide

- **Marking the mini columns**
  Our columns have a larger fringe. This zone is very well suited to mark the column, if necessary.
  This larger fringe provides several additional benefits during the handling of the columns. First, it is easier to hold them and thereby it is safer to transfer the columns from the centrifuge to the rack.
  Second, it happens quite often that you get into contact with the solutions inside, when you cannot hold the tubes with its small fringe in your fingers. Therefore, your fingers as well as the solution may become contaminated.
  You have to wear gloves while handling these columns.
  The collection tube has a very special shape. This shape makes it more stable, which is advantageous for multiple reuse.

- **Residual DNA of a previous preparation detectable by PCR**
  Relief: Extend the incubation with RG1. For very sensitive samples, the duration of the incubation may be extended up to 24 hours without damaging the silica materials. We made the experience that we have never been able to detect any residual DNA contaminations after an incubation of 60 minutes with RG1.

- **Discolouring of silica columns**
  In some cases after multiple regeneration cycles, we observed a yellow to brownish colour on the membranes of the silica materials.
  This colour originates from small insoluble particles of the colour indicator. It does not impair the functionality of the columns. In most cases it can be removed by washing the column with 50 mM EDTA (pH 8.0).
  Apply the same volume of the EDTA solution as for RG1 and RG2. Incubate the column with EDTA after removal of RG1 and remove it by centrifugation, vacuum or gravity.

- **Prezipitate of RG1 on the column**
  If you incubate a column with RG1 at temperatures below 20°C over night (e.g. the air condition cools down below 20°C) ingredients of RG1 may precipitate. Remove this precipitate by washing the columns twice with RG2 at 40°C.

- **Reduced binding capacity after long-term storage**
  In very few cases, we observed a reduced binding capacity of regenerated columns for new DNA after long-term storage (in our case after approximately 2 months). If you treat the column with RG1 and RG2 just before reusing them, this will overcome the problem and the full binding capacity is recovered. Therefore, don't throw any used columns away! Repeating the treatment of the columns with RG1 and RG2 will overcome most problems. We never lost a single column since introducing MAXXBOND.

- **Minipreps may plug columns**
  Loose parts of the pellets may plug columns, when the clear supernatant of the precipitation step (after the centrifugation) hasn't been transferred by a pipettor but by pouring it directly on the column.
  Therefore, we recommend to transfer the DNA-containing supernatant by a pipettor.
  Tests have shown that the quality of the lysis buffer and the neutralisation / precipitation solutions is critical. If these solutions haven't been optimized for clear supernatants, columns may plug. Usage of our MAXXMORE reagents will overcome this problem.