



PRODUCT INFORMATION SHEET

IMMOBILIZED PROTEINASE K G3M

P3502

Proteinase K from *Tritirachium album*. Chromatographically purified, free of ribo- and deoxyribonucleases.

Proteinase K is an unspecific serine protease with strong proteolytic activity on denatured (in SDS) and high molecular weight native proteins. It cleaves peptide bonds mostly after the carboxyl group of N-substituted hydrophobic, aliphatic and aromatic amino acids.

G3m: 25 µg proteinase K immobilized on matrix G3m per CR-column.

0.7 mAnson units immobilized per CR-column.

This CR-column cuts at least 370 µg BSA per application.

Nr. 5 Storage buffer: 50 mM Tris/HCl, pH 7.5

Nr. 16 Reaction buffer: 50 mM Tris/HCl, 5 mM NaCl, pH 8.0

Nr. 17 Washing buffer: 50 mM Tris/HCl, 1.0 M NaCl, pH 8.0

The columns are more active in 0.1% SDS and at 40°C. Also active in PBS buffer (20 mM Na-phosphate, 150 mM NaCl at pH 7.6).

Protocol

1. Dilute delivered buffers (at least 2 ml each) with sterile doubly distilled water.

For 1 application you need:

1 ml 10x reaction buffer and 9 ml doubly distilled water

2 ml 5x washing buffer and 8 ml doubly distilled water

1 ml 10x storage buffer and 9 ml doubly distilled water

The substrate should be in reaction buffer

2. Equilibrate the CR-column with 10 ml reaction buffer.

Fill 10 ml reaction buffer into a syringe, let the reaction buffer run through the column by gravity to the upper filter. In case the buffer runs very slowly, apply pressure by a syringe.

3. Load substrate solution in reaction buffer.

Small volumes (< 70 µl): spin the CR-column 5 seconds in a benchtop centrifuge (2000 rpm are sufficient). Let the substrate solution enter the matrix material.

Larger volumes: Let the substrate solution run through the column.

Flow-rate: up to 70 µl/minute

Keep the substrate in the column for about 1 minute at room temperature. Higher turn-over is obtained when the substrate is applied to the column again or incubated for longer times.

4. Elute the product solution.

Small volumes (< 70 µl): centrifuge the product out of the column.

Larger volumes: Let the substrate run through the column and spin the residual solution out of the matrix

Notice: Molecules < 700 Dalton have to be eluted with 7 ml reaction buffer.

It does not harm the columns if they run dry.

5. Wash the column with 10 ml washing buffer.

6. Equilibrate the column with 10 ml storage buffer.

Store the column at 4°C. Never freeze a CR-column!