PRODUCT INFORMATION SHEET

Mo Bi Tec
MOLECULAR BIOLOGISCHE TECHNOLOGIE

IMMOBILIZED α -CHYMOTRYPSIN G3M # P3302

α-chymotrypsin from bovine pancreas. TLCK treated (free of trypsin activity).

α-chymotrypsin hydrolyzes peptides, amides, and esters preferentially at the carboxyl groups of hydrophobic amino acids (L-tyrosine, L-phenylalanine, and L-tryptophan but also bonds of leucyl, methionyl, asparaginyl, and glutamyl residues).

G3m: 25 µg α-chymotrypsin per CR-column immobilized on dextran.
1.4 units immobilized per CR-column.
This CR-column cuts at least 100 µg tubulin per application; it cuts 5 µg/minute BSA without SDS, but at least 45 µg/minute BSA in the presence of 0.1% SDS.

Nr. 5 Storage buffer: 50 mM Tris/HCl, pH 7.5
Nr. 5 Reaction buffer: 50 mM Tris/HCl, pH 7.5; also active in 0.1% SDS
Nr. 6 Washing buffer: 50 mM Tris/HCl, 1 M NaCl, pH 7.5

Protocol

For more details see MoBiTec-CRC-Handbook.

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!

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