PRODUCT INFORMATION SHEET

Mo Bi Tec
MOLECULAR BIOLOGISCHE TECHNOLOGIE

IMMOBILIZED PEPSIN G3M # P3122

Pepsin from *porcine mucosa*.
Pepsin is an endopeptidase. It hydrolyses proteins and peptides favorably adjacent to aromatic and dicarboxylic L-amino acid residues, preferentially phenylalanine and leucine but not next to valine, alanine, and glycine.
Pepsin is not stable above pH 6.0.
G3m: 25 µg pepsin (0.4 m Anson) immobilized on dextran per CR-column,
(1 m Anson unit is equivalent to 1 µmole of Foline-positive amino acids calculated as tyrosine released from denaturated hemoglobin per minute at 37.5°C at pH 2; this mAnson unit is equivalent to ca. 180 D-A280 units).

Storage buffer:	50 mM glycine, 10% (v/v) glycerol, pH 4.0
Reaction buffer:	20 mM Na acetate, pH 4.7.
Washing buffer:	20 mM Na acetate, 1.0 M NaCl, pH 4.7

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 1ml 100% glycerol and 8 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 < 70 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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