



**Mo Bi Tec**  
MOLECULAR BIOLOGISCHE TECHNOLOGIE

# PRODUCT INFORMATION SHEET

## IMMOBILIZED $\alpha$ -CHYMOTRYPSIN F7M

# P5301

$\alpha$ -chymotrypsin from bovine pancreas. TLCK treated (free of trypsin activity).

$\alpha$ -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, asparaginy, and glutamyl residues).

**F7m:** 1.0 mg  $\alpha$ -chymotrypsin per CR-column immobilized on polyvinyl.  
55 units immobilized per CR-column

**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:

0.25 ml 10x reaction buffer and 2.25 ml doubly distilled water

0.4 ml 5x washing buffer and 1.6 ml doubly distilled water

0.2 ml 10x storage buffer and 1.8 ml doubly distilled water

The substrate should be in reaction buffer

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

Fill 2 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 (< 80  $\mu$ l): 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 80  $\mu$ l/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 (< 80  $\mu$ l): Elute the product with 500  $\mu$ l reaction buffer.

Larger volumes: Let the substrate run through the column and elute the residual product solution with 500  $\mu$ l reaction buffer.

It does not harm the columns if they run dry.

### 5. Wash the column with 2 ml washing buffer.

### 6. Equilibrate the column with 2 ml storage buffer.

Store the column at 4°C.

Never freeze a CR-column!