

PRODUCT INFORMATION SHEET

IMMOBILIZED β -GALACTOSIDASE F7M

A5101

β -galactosidase from *Escherichia coli*.

β -galactosidase catalyzes the hydrolysis of β -D-galactoside to galactose and alcohol.

F7m: 1.0 mg β -galactosidase immobilized on polyvinyl ,
780 units immobilized per CR-column.

100 μ l of a 5% lactose solution is digested to over 80% in 60 minutes

Nr.14 Storage buffer: 50 mM Tris/HCl, pH 7.8

add DTT to the storagebuffer, final concentration: 1 mM DTT

Nr.64 Reaction buffer: 50 mM phosphate, 1 mM $MgCl_2$, pH 7.8 add β -mercaptoethanol,
to the reactionbuffer, final concentration: 10 mM

Nr.63 Washing buffer: 50 mM phosphate, 1 M NaCl, pH 7.8

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+
10 mM β -mercaptoethanol

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+
DTT (1 mM)

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 μ 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 μ 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 μ l): Elute the product with 500 μ l reaction buffer.

Larger volumes: Let the substrate run through the column and elute the residual product solution with 500 μ 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!