FT-35186B

Immobilized Trypsin

Product Description

TPCK-treated Trypsin immobilized on agarose, for easy cleavage of proteins, notably Igs for the preparation of Immunoglobulin fragments.

	Immobilized TCPK Trypsin Resin
cat.number:	35186A, 2ml 35186B, 5ml 35186C, 15ml
Matrix:	Agarose 6%, crosslinked, beaded

Storage: Store at °C. DO NOT FREEZE. (L)
Stability is > 1 year at +4°C

Applications:
- Efficient conversion of IgM into IgG-type-M (200kDa) + Fc5µ-H (340KDa), and further Fab'2 fragments or Fab-H
  - Rapid digestion of IgGs thanks to high enzyme load and activity
  - No enzyme contamination in the final preparation
- Removal of adherent cells from tissue culture flasks
- Preparing tryptic fragments for Edman degradation sequencing
- Purification soybean trypsin inhibitor

Benefits:
- Cleaves at carboxyl side of arginine and lysine residues
- Digestion conditions: pH 7.5-9.0, 37°C
- ease of separation from cleavage products
- Lowest leaching of trypsin
- High of enzyme activity and very stable gel)
- Reusable

Introduction

Uptima Trypsin-Gel is an immobilized enzyme designed for the convenient preparation of F(ab)2 fragments while avoiding contamination of the final preparation with the enzyme.

The preparation of fragments from IgG antibodies is interesting in R&D and particularly important for therapeutic and diagnostic purposes, including intravenously administered passive and active immunotherapies as well as the elimination of nonspecific, Fc receptor-mediated binding of IgG antibodies to cellular surfaces.

Trypsin is a 23.8kDa pancreatic serine endoprotease. It cleaves proteins at carboxyl side of arginine and lysine residues at pH 7.5-9.0, at 37°C. The isoelectric point of trypsin is 9.3.

Trypsin is treated with L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK) to inhibit contaminating chymotrypsin activity without affecting trypsin activity.

TPCK Trypsin is used for amino acid analysis and protein sequencing, mapping and structural studies. Our Immobilized TPCK Trypsin can replace free trypsin in many applications with the benefit to minimize autolysis, eliminate contamination of a sample with the protease and allow control of the digestion by removing the trypsin. It is also more stable against heat-induced denaturation, resulting in longer maintenance of activity.
A major benefit of the immobilized form is to allows sample cleavage and separation after treatment.
Additionnaly, the Immobilized Trypsin allows for affinity purification of Trypsin Inhibitors

Fragments of interest can be removed from undigested IgG or IgM and other fragments by suitable method. Further treatments allow to prepare several kind of fragments.

Trypsin digestion of several species of IgM was studied in 0.1M Tris.HCl, 0.2M NaCl, 0.01M $CaCl_2$, pH 8.3 at an Enzyme/Substrate ratio of 1:100 (w/w) at 55°C (see References section). The digestion of mouse IgM yielded F(ab') 2 and Fab but not (Fc)5µ (the Cµ3 and the Cµ4 domains of the mouse may be unstable). Trypsin digestion of human IgM yielded Fab and (Fc)5(degraded to subunits and peptides).

Trypsin has been reporter to prepare F(ab')2 fragments from IgG that are resistant to digestion by pepsin, e.g. sheep IgG.
Fab and Fc fragments from human and mouse IgM have been prepared in the presence of 5M urea.
See the reference literature.

Directions for use

Protocol forTrypsin digestion of proteins

Note: Optimization of Immobilized Trypsin protocol is required for specific applications. Recommended reaction conditions are pH 7.5 to 9.0 at 37ºC. The reaction rate will be increased by increasing the enzyme to protein substrate ratio and incubation temperature. For example, a typical digestion of 4 hours to overnight can be achieved using a ratio of 1:25 enzyme to protein substrate, while it is recommended to use 1:10 enzyme to protein substrate for accelerated trypsin digestion (0.5 to 1 hour).

1. Prepare the Digestion Buffer (A): 100 mM Ammonium Bicarbonate ($NH_4 HCO_3$), pH 8 (or other suitable buffer such as 100 mM Triethylamine Acetate, pH 8)

2. Wash 0.15 to 0.3 ml of the Immobilized Trypsin with 3 x 0.5 ml of digestion buffer.
Rem: the 50% slurry of gel can be pipetted during gentle mixing of gel with a wide bore pipette tip (or a tip which top has been cut).
Add 0.5 ml digestion buffer and swirl gently to mix for 1min.
Allow the resin to separate from the buffer by centrifugation (~800 – 1 000 x g for 2 - 5 minutes) or by using a serum separator and discard the buffer.
Repeat wash step then resuspend Immobilized Pepsin in 200µl of digestion buffer.

4. Prepare up to 1 mg IgG in 500µl digestion buffer A
IgG should be pure, free of other proteins or strong buffering agents. Otherwise exchanging buffer may be performed by extensive dialysis (CelluSep) against digestion buffer and eventually reconcentrate (to 10 mg/ml).

5. Add the prepared IgG (1mg/0.2ml) to washed resin from step 2 (0.2ml gel suspension).
Incubate the reaction mixture with continuous agitation in a shaking water bath for 2-18 hours at 37°C.
Note: Incubation duration should be determined for each antibody (10µl sol. can be taken and analysed later for determining the optimal duration). See more information at note [A]).
Alternately, the quantity of gel may be increased when fragmentation was found insufficient.

6. Separate the Immobilized Trypsin gel from the digestion mixture as noted in step 2 (filtration or centrifugation).
Note: Maximum fragment recovery can be obtained by washing Immobilized Trypsin with suitable buffer. Add wash to the recovered digest .
Retain supernatant in a new tube as your trypsin - digested protein sample and check for pH.
Buffer may be exchanged for downstream uses, purification, further cleavage/reduction, analysis…

9. Trypsin agarose should be cleaned with the digestion buffer, and stored with 0.05% NaN3 and 50% Glycerol.

[C] References

1. Trypsin activity

Kostka, V. and Carpenter, F.H.

J Biol Chem (1964) 239:1799-1803

2. Trypsin for Ig fragmentations

Davies, M.E., et al. (1978). Preparation of antibody fragments: Conditions for proteolysis compared by SDS - gel electrophoresis and quantitation of antibody yield . J ImmunolMeth 21: 305 -15.

Matthew, W.M. and Reichardt, L.F. (1982). Development and application of an efficient procedure for converting mouse IgM into small, active fragments . J Immunol Meth 50: 239 -53.

Beeale, D. and VanDort, T. (1982). A comparison of the proteolytic fragmentation of the immunoglobulin M from several mammalian species . Comp Biochem Physiol 71B(3): 475 -82.

Beale, D. and Hopley, J. (1983). A comparison of the fragmentation of the different species of mammalian immunoglobulin M by trypsin in urea. Comp Biochem Physiol 76B(2): 385 -9.

Other Information

Related products:

2-MEA HCl (mercaptoethylamine, cysteamine) #XMB030, DTT #054721 and TCEP #UP242214
- used to fragment IgM in IgG-type(Mouse, Human - (200KDa) then r IgG(110KDa)
Iodoacetamide #02048n
Protein A-Agarose (#UP49981, #UP90467) and Protein G- agarose (#UP75196).
Immobilized Pepsin-Agarose #49978B[FT]

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