

Immobilized Pepsin

20343

0121.3

Number	Description
20343	<p>Immobilized Pepsin, 5mL settled gel, contains sufficient material to generate F(ab')₂ fragments from up to 20 samples containing up to 10mg of IgG</p> <p>Supplied: 50% slurry containing 50% glycerol in 0.1M sodium acetate; pH 4.5, and 0.05% sodium azide as a preservative</p> <p>Support: 6% cross-linked beaded agarose</p>

Storage: Upon receipt store product at 4-8°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific Immobilized Pepsin allows efficient generation of F(ab')₂ from IgG. Pepsin is a nonspecific endopeptidase that is active only at acid pH and irreversibly denatured at neutral or alkaline pH. Immobilized Pepsin is advantageous because the digestion can be immediately stopped by simply removing the gel from the IgG. Digestion by pepsin normally produces a F(ab')₂ fragment and numerous small peptides of the Fc portion (Figure 1). The resulting F(ab')₂ fragment is composed of two disulfide-connected Fab units. The Fc fragment is extensively degraded, and its small fragments can be separated from F(ab')₂ by dialysis, gel filtration or ion exchange chromatography.

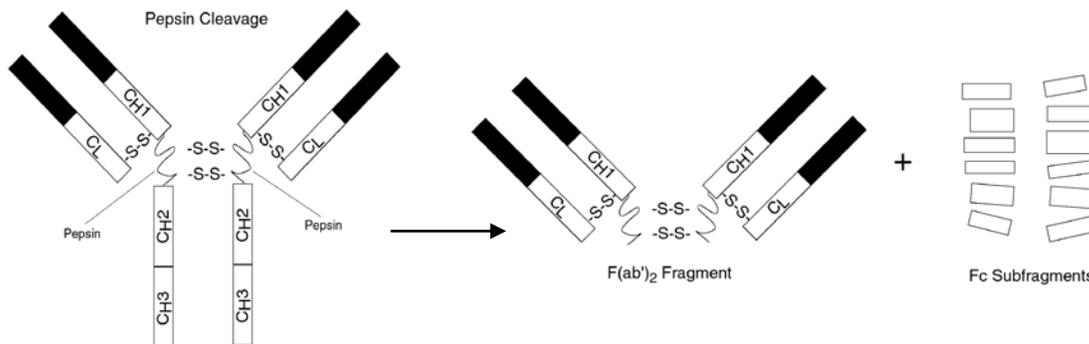


Figure 1. Pepsin digestion of IgG. Digestion normally produces a F(ab')₂ fragment and an extensively degraded Fc portion.

Additional Materials Required

- Shaking water bath capable of maintaining 37°C
- Pipettors capable of accurately dispensing 0.25mL, 0.5mL, 1mL and 3mL
- Sodium acetate, trihydrate, ACS Reagent Grade

Note: Immobilized Protein A Columns (Product No. 20356) can be used to separate undigested IgG from F(ab')₂ fragments contaminated with small Fc fragments that cannot bind to Protein A. To remove small Fc fragments from the F(ab')₂ fragments, perform dialysis (50K MWCO), gel filtration or ion exchange chromatography.

Material Preparation

IMPORTANT: proper sample preparation is essential for successful fragment generation using this kit.

Digestion Buffer	Dissolve 2.72g of sodium acetate, trihydrate in 1L of ultrapure water to prepare a 20mM solution. Adjust pH to 4.5. Store unused Digestion Buffer at 4°C. Discard buffer if there is any evidence of microbial or fungal growth during storage.
Immobilized Pepsin Equilibration	Gently swirl vial to obtain an even suspension. Using a wide bore or cut pipette tip, place 0.25mL of the 50% slurry (i.e., 0.125mL of settled gel) of Immobilized Pepsin into a 15mL tube and add 4.0mL of Digestion Buffer. Separate Immobilized Pepsin from buffer by centrifugation (e.g., centrifuge at 1000 × g for 1-5 minutes) and discard buffer. Repeat this wash procedure. Resuspend the Immobilized Pepsin in 0.5mL of Digestion Buffer.
IgG Sample	If IgG is purified, salt free and lyophilized, dissolve up to 10mg of IgG in 1.0mL of Digestion Buffer. If IgG is in solution, dialyze IgG against the Digestion Buffer. Concentrate IgG to ~10mg/mL. A Thermo Scientific Pierce Protein Concentrator (Product No. 89886A) may be used for performing the buffer exchange and for concentrating.

Procedure for Generating F(ab')₂ Fragments

Note: For best results, perform preliminary studies to determine optimal incubation times for each IgG species and subclass.

1. Add 1.0mL of the prepared IgG to the tube containing the equilibrated Immobilized Pepsin.
2. Incubate IgG for the appropriate time (4 hours for human IgG) at 37°C in a high speed, shaking water bath; end-over-end mixer or tabletop rocker. Maintain constant mixing of gel during incubation.
3. Separate digest from the Immobilized Pepsin by centrifugation (e.g., centrifuge at 1000 × g for 1-5 minutes) or by using a resin separator. Decant crude digest into a new tube.
4. For maximum fragment recovery, wash Immobilized Pepsin with 1.5mL of 10mM Tris•HCl, pH 7.5. Add wash to crude digest. Total sample volume should be 3.0mL. Discard used Immobilized Pepsin.

Note: Immobilized Protein A Columns can be used to separate undigested IgG from F(ab')₂ fragments contaminated with small Fc fragments that cannot bind to Protein A. To remove small Fc fragments perform dialysis (50K MWCO), gel filtration or ion exchange chromatography.

Related Thermo Scientific Products

20356	Pierce® Protein A Columns , 5 × 1mL settled gel pre-packed columns
44985	Fab Preparation Kit
44980	IgG₁ Fab and F(ab')₂ Preparation Kit
44988	F(ab')₂ Preparation Kit
23225	BCA Protein Assay Kit , sufficient to perform 500 standard tube assays
25200-44	Precise™ Protein Gels (see catalog or web site for a complete listing)

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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