INSTRUCTIONS

# Immobilized Pepsin

## 20343

0121.3

| Number | Description |
|---|---|
| **20343** | **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 |
| | Supplied: 50% slurry containing 50% glycerol in 0.1M sodium acetate; pH 4.5, and 0.05% sodium azide as a preservative |
| | Support: 6% cross-linked beaded agarose |
| | **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.

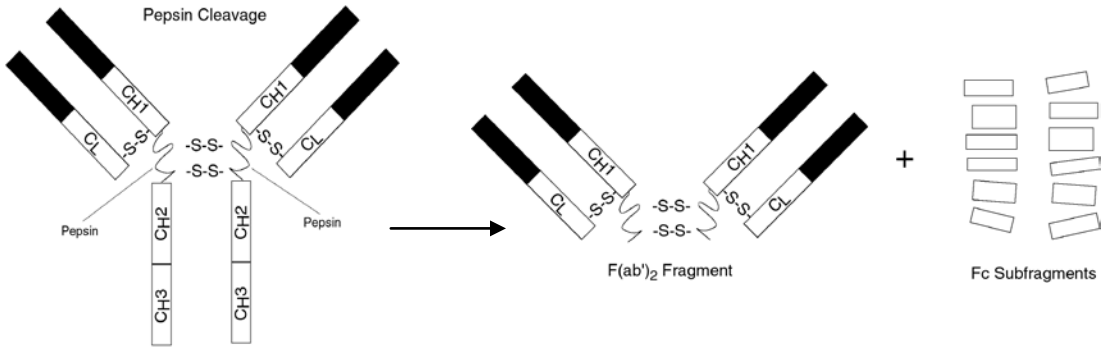


**Figure 1. Pepsin digestion of IgG.** Digestion normally produces a $F(ab')_2$ fragment and an extensively degraded Fc portion.

## 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.


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## Material Preparation

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

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| 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.<br>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

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| **20356** | **Pierce® Protein A Columns,** 5 × 1mL settled gel pre-packed columns |
| **44985** | **Fab Preparation Kit** |
| **44980** | **IgG$_1$ 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) |



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