G-Biosciences, St Louis, MO. USA ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

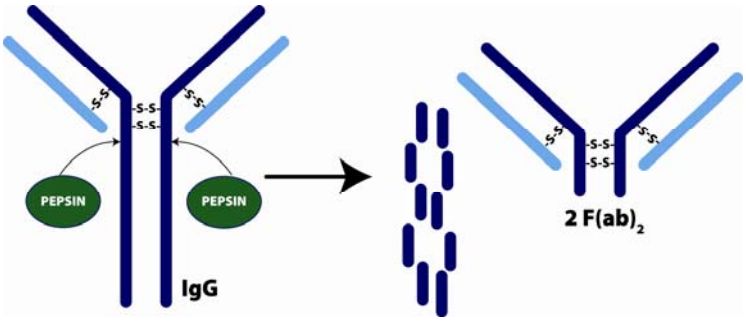
A Geno Technology, Inc. (USA) brand name

Immobilized Pepsin

For the Generation of F(ab)$_2$ Fragments from IgG

INTRODUCTION

Pepsin is a proteolytic enzyme that is routinely used for the generation of F(ab)$_2$ fragments from immunoglobulin G (IgG). The pepsin has the ability to cleave the heavy chains near the hinge region (see figure). One or more of the disulfide bonds that join the heavy chains in the hinge region are preserved, so the two Fab regions of the antibody remain joined together, yielding a divalent molecule (containing two antibody binding sites), hence the designation F(ab)$_2$. The light chains remain intact and attached to the heavy chain, whereas the Fc fragment is digested into small peptides.



The Immobilized Pepsin offers the distinct advantage of eliminating enzyme contamination of the F(ab)$_2$ fragments.

The F(ab)$_2$ fragments can be purified from undigested IgG with Immobilized Protein A (Cat. # 786-283) and can be further purified from the small Fc fragments by dialyze with a 50kDa MWCO membrane (Tube-O-DIALYZER™ (Cat. # 786-614, 786-619, 786-624)) or by gel filtration.

Supplied as a 50% slurry in 50% glycerol, 0.1M sodium acetate, pH4.4 with sodium azide as a preservative.

KIT COMPONENTS

Cat. #	Description	Size
786-791	Immobilized Pepsin	5ml resin

STORAGE CONDITIONS

Shipped at ambient temperature. Upon receipt store at 4°C, do NOT freeze.

IMPORTANT INFORMATION

- **Support**: 6% Cross-linked Agarose

ADDITIONAL COMPONENTS

- Shaking 37°C Waterbath
- Digestion Buffer (20mM Sodium acetate, pH4.5), store at 4°C
- Purified, lyophilized IgG or ≥20mg/ml IgG solution
- Wash Buffer: 10mM Tris.HCl, pH7.5

PREPARATION BEFORE USE

1. ***Antibody Preparation:*** If using an IgG solution, dialyze against the Sample Buffer and concentrate to ~10mg/ml.
   NOTE: *We recommend using Tube-O-DIALYZER™ (Cat. # 786-610 to 786-624) for dialysis to ensure no loss of antibody.*

2. ***Resin Preparation:*** Suspend the resin by gently shaking and inverting the resin. Transfer 0.25ml of the slurry to a 15ml tube with a wide bore pipette tip. Equilibrate the resin with the addition of 4ml Digestion Buffer. Centrifuge at 1,000g for 2-5minutes to pellet the resin, remove the Digestion Buffer. Repeat the wash with a further 4ml Digestion Buffer. Resuspend the washed resin in 0.5ml Digestion Buffer.

PROCEDURE

1. Dissolve ≤10mg pure, lyophilized IgG in 1ml Digestion Buffer.

2. Add 1ml IgG sample to the Immobilized Pepsin. Seal the tube and incubate at 37°C in a high speed shaking waterbath for the indicated time:
    a. For rabbit, human and mouse IgG_1 incubate for 12-24 hours to overnight.
    b. For all other IgG; incubate for 6 hours to overnight.

3. Centrifuge at 1,000g for 5 minutes to pellet the resin and collect the supernatant.
    NOTE: *For maximum recovery, wash the resin with 1.5ml 10mM Tris.HCl, pH7.5 and add wash to the supernatant.*

4. To separate the $F(ab)_2$ fragments from the undigested IgG, use Immobilized Protein A (Cat. # 786-283) or ion exchange. To separate $F(ab)_2$ fragments from the small Fc fragments dialyze with a 50kDa MWCO membrane or use gel filtration.

RELATED PRODUCTS

I. ***Immobilized Papain*** (Cat. # 786-790): *For the digestion of IgG molecules to generate Fab and Fc fragments.*

II. ***Immobilized Protein A*** (Cat. # 786-283): *For the purification of IgG molecules or separation of Fab fragments from Fc fragments.*

III. ***Tube-O-DIALYZER***™ (Cat. # 786-610 to 786-624): *Tube format micro dialysis devices for 20-250µl (Micro) or 0.2-2.5ml (Medi) volumes. Available in 1, 4, 8, 15 and 50kDa MWCO.*

For additional related products, visit www.GBiosciences.com.

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