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A Geno Technology, Inc. (USA) brand name

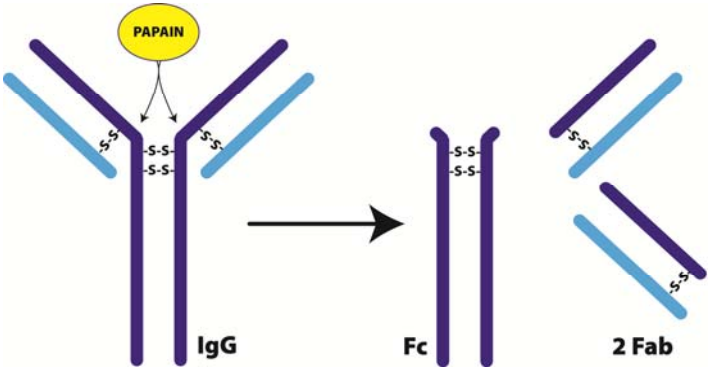
Immobilized Papain

For the Generation of Fab & Fc Fragments from IgG

(Cat. # 786-790)

INTRODUCTION

Papain is a cysteine protease enzyme (EC 3.4.22.2) that has the endopeptidase activity to cleave immunoglobulin G molecules in the hinge region. This cleavage results in the generation of three ~50kDa fragments; two Fab domains and a Fc domain. The papain-digested antibody is unable to promote agglutination, precipitation, opsonization, and lysis. The Fab and Fc fragments can be further separated with either Immobilized Protein A (Cat. # 786-283) or ion exchange chromatography.



Immobilized Papain is a convenient reagent for producing Fab and Fc fragments as it avoids the need to remove the papain enzyme after digestion.
Supplied as a 50% slurry in 50% glycerol, 0.1M sodium acetate, pH4.4 with sodium azide as a preservative.

ITEM(S) SUPPLIED (Cat. # 786-790)

Description	Size
Immobilized Papain	5ml resin

STORAGE CONDITIONS

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

IMPORTANT INFORMATION

- **Activity:** ≥15-40 BAEE units/ml resin (*One* unit *will hydrolyze 1.0 µmole of BAEE per minute at pH6.2 at 25°C*)
- **Capacity:** 250µg papain/ml resin
- **Support**: 6% Cross-linked Agarose

ADDITIONAL ITEM(S) REQUIRED

- Sample Buffer (20mM Sodium phosphate, 5mM EDTA, pH7.0)
- Cysteine.HCl
- Purified, lyophilized IgG or ≥20mg/ml IgG solution
- Wash Buffer: 10mM Tris.HCl, pH7.5

PREPARATION BEFORE USE

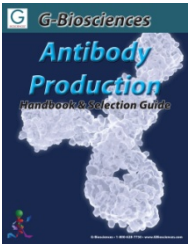
1. ***Antibody Preparation:*** If using an IgG solution, dialyze against the Sample Buffer and concentrate to ~20mg/ml.
   NOTE: *We recommend using Tube-O-DIALYZER™ (Cat. # 786-610 to 786-624) for dialysis to ensure no loss of antibody.*
2. ***Digestion Buffer:*** Immediately prior to digestion, add Cysteine.HCl to the Sample Buffer to give a final concentration of 20mM and adjust the pH to pH7.0. *Use 35mg Cysteine.HCl for every 10ml Digestion Buffer.*
3. ***Resin Preparation:*** Suspend the resin by gently shaking and inverting the resin. Transfer 0.5ml 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.

PROTOCOL

1. Dissolve ≤10mg pure, lyophilized IgG in 1ml Digestion Buffer or add 0.5ml dialyzed, concentrated IgG to 0.5ml Digestion Buffer to give ~10mg/ml.
2. Add 1ml IgG sample to the Immobilized Papain. 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 6 hours to overnight.
   b. For all other IgG; incubate for 4 hours to overnight.
3. Add 1.5ml Wash Buffer direct to the digest and then centrifuge at 1,000g for 2-5 minutes to pellet the resin and collect the supernatant.
4. To separate the Fab fragments from the Fc fragments, use Immobilized Protein A (Cat. # 786-283) or ion exchange. Do not use Protein G as Fab fragments, as well as Fc fragments have some affinity for Protein G.

RELATED PRODUCTS

Download our Antibody Production Handbook.



http://info.gbiosciences.com/complete-Antibody-Production-handbook

For other related products, visit our website at www.GBiosciences.com or contact us.

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