Protocol

TD-P Revision 2.0

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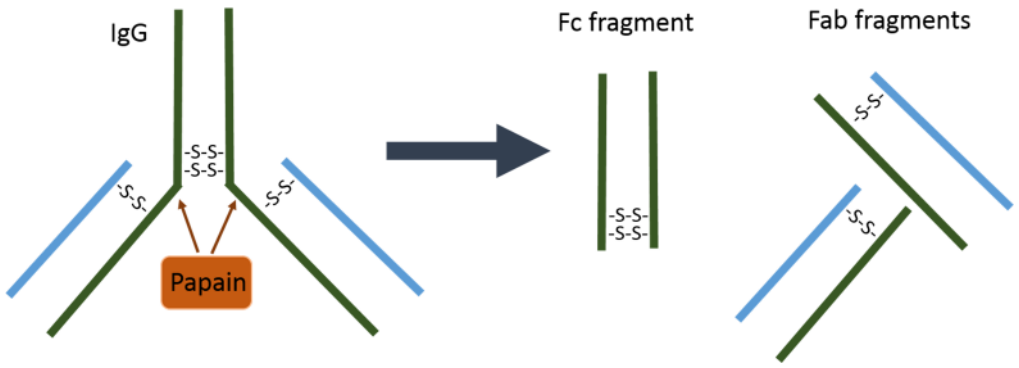
Generation of Fab & Fc Fragments from IgG

Utilizing Immobilized Papain

Introduction

Papain is a cysteine protease enzyme from papaya latex that has a wide variety of activities including endopeptidases, aminopeptidases, dipeptidyl peptidases and enzymes with both exo- and endo-peptidase activity. In particular, papain cleaves immunoglobulin G (IgG) molecules near the hinge region of the antibody, resulting in three similar, ~50 kDa, fragments; an Fc domain and two monovalent Fab domains. This papain-digested antibody is therefore unable to promote agglutination, precipitation, opsonization or lysis. The Fc fragment can then be used to determine the specificity of the Fc receptors without any typical antigen binging interference while the Fab fragments can be used in immunohistochemical studies.



Materials

- Immobilized Papain (6% crosslinked agarose resin)

Required, but not supplied

- Sample Buffer: 20mM Sodium phosphate, 5mM EDTA, pH 7.0
- Wash Buffer: 10mM Tris-HCl, pH 7.5
- L-Cysteine Hydrochloride
- Purified, lyophilized IgG or ≥20 mg/ml IgG solution

Method

Antibody Preparation

1. If using an IgG solution, dialyze against the Sample Buffer and concentrate to ~20 mg/ml.

Gold Biotechnology
St. Louis, MO
Ph: (800) 248-7609
Web: www.goldbio.com
Email: contactgoldbio86@goldbio.com

Digestion Buffer Preparation

2. Immediately prior to digestion, add L-Cysteine HCl to the Sample Buffer to give a final concentration of 20mM and adjust to pH 7.0. Use 35 mg L-Cysteine HCl for every 10 ml Digestion Buffer.

Resin Preparation

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

Generation of Fragments

4. Dissolve ≤10 mg pure, lyophilized IgG in 1 ml Digestion Buffer or add 0.5 ml dialyzed, concentrated IgG to 0.5 ml Digestion Buffer to give ~10 mg/ml concentration.

5. Add 1 ml IgG sample to the Immobilized Papain Agarse Resin. 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, incubate for 6 hours to overnight.
    b. For all other IgG, incubate for 4 hours to overnight.

6. Add 1.5 ml Wash Buffer direct to the digest and then centrifuge at 1,000 x g for 2-5 minutes to pellet the resin and collect the supernatant.

7. To separate the Fab fragment from the Fc fragment, use Protein A Agarose Resin (GoldBio Catalog # P-400) or use ion exchange.

Note: Do not use Protein G, as both Fab and Fc fragments have some affinity for Protein G.