Protocol

TD-P Revision 2.0

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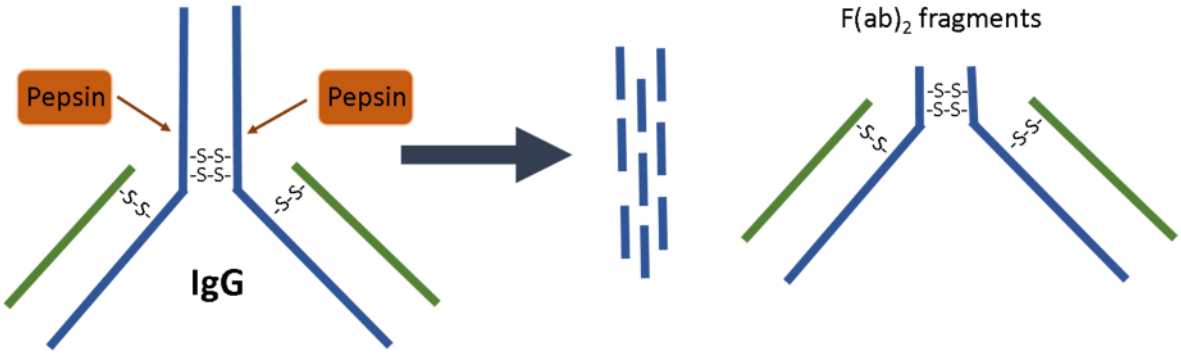
Generation of F(ab)2 Fragments from IgG

Utilizing Immobilized Pepsin

Introduction

Pepsin is a proteolytic enzyme that is routinely used for the generation of $F(ab)_2$ fragments from immunoglobulin G (IgG). 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. These fragments can be purified from undigested IgG with immobilized protein A and can be further purified from the small Fc fragments via gel filtration.

Materials

- Immobilized Pepsin (6% Cross-linked agarose) (GoldBio Cat # I-030)
- Shaking 37°C water bath
- Digestion buffer (20mM Sodium acetate, at pH 4.5), store at 4°C
- Purified, lyophilized IgG or ≥ 20 mg/mol IgG solution
- Wash Buffer: 10mM Tris HCl, at pH 7.5 (GoldBio Cat # T-095)

Storage and Handling

- Immobilized Pepsin is shipped at ambient temperature. Upon receipt store at 4°C, do NOT freeze.

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

- Supplied as a 50% slurry in 50% glycerol, 0.1M sodium acetate, at pH 4.4 with sodium azide as a preservative.

Method

Antibody Preparation

1. If using an IgG solution, dialyze against the Sample Buffer and concentrate to ~10 mg/ml.

Resin Preparation

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

Generation of Fragments

8. Dissolve ≤ 10 mg pure, lyophilized IgG in 1 ml Digestion Buffer.
9. Add 1 ml IgG sample to the Immobilized Pepsin. Seal the tube and incubate at 37°C in a high speed shaking water bath 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.
10. Centrifuge at 1,000 x g for 5 minutes to pellet the resin and collect the supernatant.
11. To separate the $F(ab)_2$ fragments from the undigested IgG, use Protein A Agarose Resin (GoldBio Catalog # P-400) or use ion exchange. To separate $F(ab)_2$ from the small Fc fragments use gel filtration with 6% Agarose Beads, Fine (GoldBio Cat # A-174) or dialyze with a 50kDa MWCO membrane.

Associated Products

- Immobilized Pepsin (6% Cross-linked agarose) (GoldBio Cat # I-030)
- Protein A Agarose Resin (GoldBio Catalog # P-400)

- Tris HCl (GoldBio Cat # T-095)
- 6% Agarose Beads, Fine (GoldBio Cat # A-174)