Protocol

TD-P Revision 2.0

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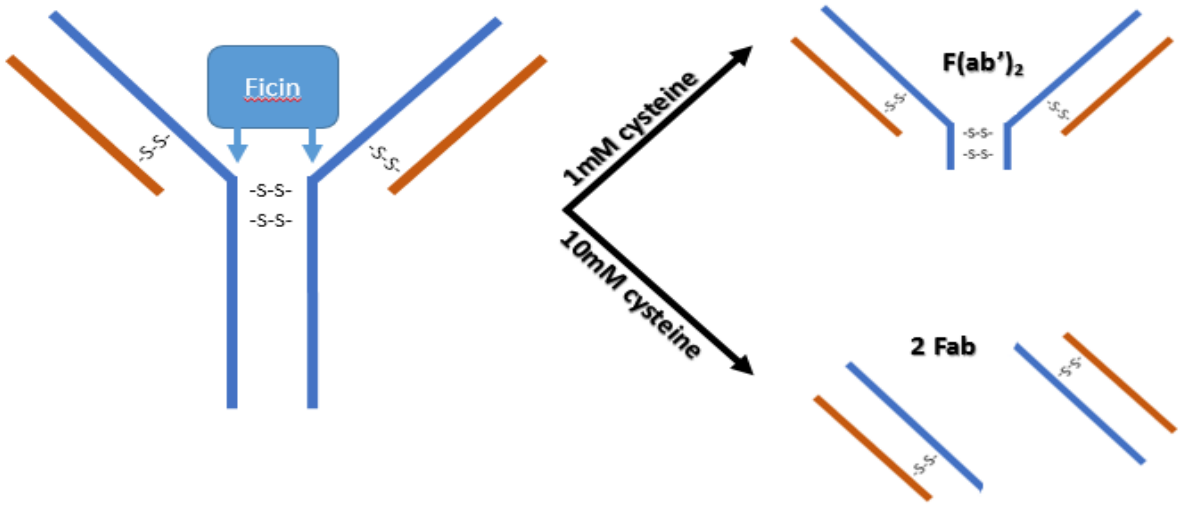
Generation of Fab and Fc Fragments from Mouse IgG1

Utilizing Immobilized Ficin

Introduction

Ficin (or Ficain) (~25 kDa) is a cysteine protease enzyme (EC 3.4.22.3) isolated from fig latex that has endopeptidase activity and can cleave immunoglobulin G molecules in the hinge region. Ficin has an effective range of pH 4.0-9.5 with an optimal pH of 6.5 and cleaves bonds that involve uncharged or aromatic amino acids. Ficin is typically used to cleave mouse IgG_1, which is uncleavable by papain and pepsin. In the presence of 1mM or 10mM cysteine, ficin generates $F(ab')_2$ and Fab fragments respectively. The Fab and $F(ab')_2$ fragments can be separated from whole IgG and Fc with either Protein A Agarose Resin or ion exchange chromatography. Immobilized ficin is a convenient reagent for producing Fab and $F(ab')_2$ fragments as it avoids the need to remove the ficin enzyme after digestion.



Supplied as a 30% slurry in 50% glycerol, 0.1M sodium acetate, pH 4.4 with sodium azide as a preservative.

Materials

- Immobilized Ficin (6% Cross-linked Agarose)

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

Required, but not supplied

- 100mM Citrate Buffer, pH 6.0
- L-Cysteine Hydrochloride
- EDTA Disodium (GoldBio Catalog # E-210)
- Purified, lyophilized IgG or ≥20 mg/ml IgG solution
- Wash Buffer: 10mM Tris-HCl at pH 7.5

100mM Citrate Buffer, pH 6.0

1. Add 2.10 g of citric acid monohydrate (mw. 210.14) to 100 ml dH_2O for a 100mM solution.
2. Add 2.94 g of trisodium citrate, dihydrate (mw. 294.10) to 100 ml dH_2O for a 100mM solution.
3. Combine 11.5 ml of 100mM citric acid monohydrate solution to 88.5 ml of 100mM trisodium citrate solution to make a 100mM citrate buffer at pH 6.0.

Activation Buffer

1. Immediately prior to digestion, add 0.93 g of EDTA Disodium (50mM, mw. 372.24) to 50 ml of 100mM Citrate buffer, pH 6.0 and dissolve.

2. To Activation Buffer, add either:

a. For $F(ab')_2$ digestion: 8.78 mg Cysteine-HCl (1mM, mw. 175.63)
b. For Fab digestion: 87.82 mg Cysteine-HCl (10mM, mw. 175.63)

Antibody Preparation

1. Prepare 0.5-10.0 mg/ml of mouse IgG_1 in 1 ml Activation Buffer (see Step 5 for IgG concentration vs. incubation time).

Method

Resin Preparation

1. For Column Digestion: Suspend the resin by gently shaking and inverting the resin. Transfer 2-4 ml of the slurry (1-2 ml resin) to a suitable column with a wide bore pipette tip. Equilibrate the resin with the addition of 20 ml Activation Buffer. Allow the Activation Buffer to pass through the column by gravity flow but do not allow the column to run dry.

   For Suspension Digestion**:** Suspend the resin by gently shaking and inverting the resin. Transfer 2-4 ml of the slurry (1-2 ml resin) to a 50 ml tube with a wide bore pipette tip. Centrifuge at 1,000 x g for 1 minutes to pellet the resin, then remove the majority of the perseverative solution. Equilibrate the resin with the addition of 20 ml Activation

Buffer. Centrifuge at 1,000 x g for 1 minutes to pellet the resin, then remove the Activation Buffer.

Generation of Fragments

2. Add the 1 ml IgG sample to the activated Immobilized Ficin. For column digestion, add an additional 0.25 ml Activation Buffer to the column to ensure sample fully enters the resin. Seal the tube/column and incubate while shaking at 37°C.
    a. For 0.5-10 mg/ml Mouse IgG_1 Fab fragments, incubate for 3-5 hours.
    b. For 0.5-3 mg/ml Mouse IgG1 $F(ab')_2$ fragments, incubate for 20 hours.
    c. For 3-10 mg/ml Mouse IgG_1 $F(ab')_2$ fragments, incubate for 40 hours.

3. For suspension digestion, centrifuge at 1,000 x g for 1 minutes and collect the supernatant (containing the fragments). For column flow through, use 3-4 ml of a Protein A binding buffer (i.e. sodium phosphate (25mM), pH 7.0 or Tris (50mM), pH 7.0) to thoroughly elute IgG fragment and help with the subsequent binding of any undigested IgG and Fc fragments to Protein A.

4. To separate the Fab fragments from the Fc fragments, use Protein A Agarose Resin (GoldBio Catalog # P-400), or you may also use ion exchange.

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