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A Geno Technology, Inc. (USA) brand name

Immobilized Ficin

For the Generation of Fab & Fc Fragments from Mouse IgG_1

(Cat. # 786-793)

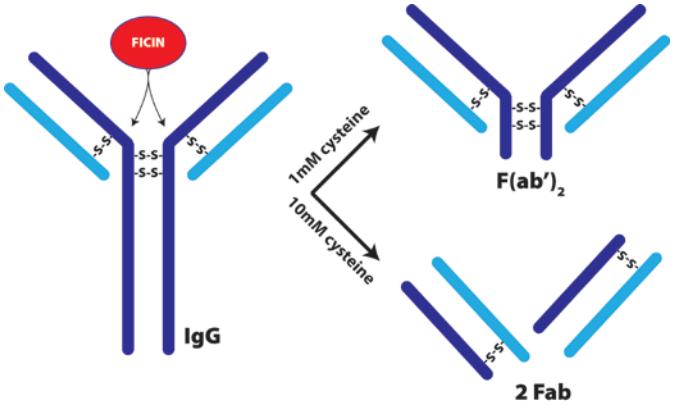
INTRODUCTION

Ficin (or Ficain) (~25,000Da) is a cysteine protease enzyme (EC 3.4.22.3) isolated from fig latex is that has the endopeptidase activity to cleave immunoglobulin G molecules in the hinge reason. Ficin has an effective range of pH4-9.5 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 as this are difficult to cleave with 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 Immobilized Protein A (Cat. # 786-283) 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, 10mM sodium tetrathionate, 2mM EDTA, pH7.0.



ITEM(S) SUPPLIED

Cat. #	Description	Size
786-793	Immobilized Ficin	5ml resin

STORAGE CONDITIONS

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

IMPORTANT INFORMATION

- **Activity:** 1-1.5mg ficin/ml of resin
- **Support**: 6% Cross-linked Agarose

ADDITIONAL COMPONENT(S) REQUIRED

- Cysteine.HCl
- EDTA
- Sample Buffer: 0.1M Citrate buffer, pH6.0
- Purified, lyophilized IgG or ≥20mg/ml IgG solution
- Wash Buffer: 10mM Tris.HCl, pH7.5

PREPARATION BEFORE USE

1. ***10X F(ab')$_2$ Digestion Buffer:*** Immediately prior to digestion, add 18.6mg EDTA to 1ml 0.1M Citrate buffer, pH6.0 and once dissolved add 1.8mg Cysteine.HCl to prepare the 10X concentrated buffer.
2. ***10X Fab Digestion Buffer:*** Immediately prior to digestion, add 18.6mg EDTA to 1ml 0.1M Citrate buffer, pH6.0 and once dissolved add 18mg Cysteine.HCl to prepare the 10X concentrated buffer.
3. ***Ficin Activation Buffer:*** Dilute the appropriate 10X Digestion Buffer in 0.1M Citrate buffer, pH6.0. Add 1ml Digestion Buffer to 9ml 0.1M Citrate buffer, pH6.0.
4. ***Antibody Preparation:*** If using an IgG solution, dialyze against the Sample Buffer and concentrate to ~0.5-10mg/ml. Add 100µl appropriate Digestion Buffer to each ml of antibody
   NOTE: *We recommend using Tube-O-DIALYZER™ (Cat. # 786-610 to 786-624) for dialysis to ensure no loss of antibody.*

5a ***Resin Preparation (Column Digestion):*** Suspend the resin by gently shaking and inverting the resin. Transfer 2-4ml of the slurry (1-2ml resin) to a suitable column with a wide bore pipette tip. Equilibrate the resin with the addition of 20ml Ficin Activation Buffer. Allow the Ficin Activation Buffer to pass through the column by gravity flow.

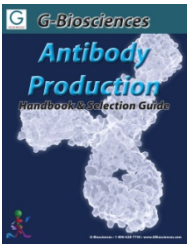
5b ***Resin Preparation (Suspension Digestion):*** Suspend the resin by gently shaking and inverting the resin. Transfer 2-4ml of the slurry (1-2ml resin) to a 50ml tube o with a wide bore pipette tip. Equilibrate the resin with the addition of 20ml Ficin Activation Buffer. Centrifuge at 1,000g for 2-5minutes to pellet the resin, remove the Digestion Buffer.

PROCEDURE

1. Add the 1.1ml IgG sample to the activated Immobilized Ficin. Add an additional 0.25ml Activation Buffer to the column to ensure sample fully enters the resin. Seal the tube/column and incubate at 37°C in a high speed shaking waterbath for the indicated time:
    a. For Mouse IgG_1 Fab fragments incubate for 3-5 hours
    b. For Mouse IgG_1 $F(ab')_2$ fragments incubate for 20 hours when using 0.5-3mg/ml antibody
    c. For Mouse IgG_1 $F(ab')_2$ fragments incubate for 40 hours when using 3-10mg/ml antibody
2. Centrifuge and collect the fragment containing supernatant or the column flow-through.
3. 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 Purification Handbook.



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

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