INSTRUCTIONS

Immobilized Ficin

44881

0638.4

Number	Description
44881	**Immobilized Ficin,** 5mL of settled resin Activity: ~1.2mg/mL of settled resin Support: Crosslinked, 6% beaded agarose Supplied: 33% slurry (i.e., 5mL of settled resin is equal to 15mL of slurry)

Introduction

The Thermo Scientific Immobilized Ficin specifically cleaves mouse IgG_1 into $F(ab')_2$ or Fab fragments in the presence of 4mM or 25mM cysteine, respectively. Fragment generation from other species and isotypes may also be possible through modification of the cysteine concentration and other digestion parameters.

Ficin, isolated from fig latex, is a sulfhydryl protease with a similar active site to bromelain and papain; however, yields and immunoreactivity are better when digesting mouse IgG_1 with ficin.[1,2] Ficin acts on bonds involving uncharged and aromatic amino acids.[3] Ficin (25K) is effective at pH 4-9.5 with an optimum pH of 6.5.[4]

Ficin immobilization enhances stability against denaturation, heat and autolysis. Immobilization also eliminates the potential for antibody-enzyme adducts that result in continued sample digestion.[5] Additionally, immobilization allows ficin to be reused.

Additional Materials

- Test tubes or Thermo Scientific Pierce Resin Separators (Product No. 69710)
- 37°C incubator
- Pipettes capable of dispensing 10-5000µL
- Empty columns (e.g., Product No. 89897) for static digestion or a mixer/shaker for suspension digestion
- Spectrophotometer capable of measuring at 280nm
- Mouse IgG_1 Mild Elution Buffer (Product No. 21034) or another low-salt buffer at pH 6-7
- Protein A Agarose (Product. No. 20333)
- IgG Binding Buffer (Product No. 21001)
- IgG Elution Buffer (Product No. 21004) or 0.1M glycine, pH 2.8
- 50% glycerol, 50mM phosphate (or other buffer), 10mM EDTA, pH 7 for Immobilized Ficin storage
- EDTA (molecular weight = 372.2)
- Cysteine•HCl (molecular weight = 175.64), (Product No. 44889)

Material Preparation

$F(ab')_2$ Stock Digestion Buffer	10X Stock Digestion Buffer (50mM EDTA, 40mM cysteine): Dissolve 18.6mg EDTA in 1mL of 0.1M citrate buffer, pH 6.0. Add 7mg cysteine to the EDTA/citrate buffer.
Fab Stock Digestion Buffer	10X Stock Digestion Buffer (50mM EDTA, 250mM cysteine): Dissolve 18mg EDTA in 1mL of 0.1M citrate buffer, pH 6.0. Add 43.9mg cysteine to the EDTA/citrate buffer. Adjust pH to 6.0.
Ficin Equilibration Buffer	Add 1 part of Fab or $F(ab')_2$ Stock Digestion Buffer to 10 parts 0.1M citrate buffer, pH 6.0 or other low-salt buffer at pH 6-7 (e.g., 50mM phosphate, pH 6.0 with < 100mM sodium chloride).
Mouse IgG_1	Prepare 1mL of mouse IgG_1 (see Table 1 for appropriate concentration) in 0.1M citrate buffer at pH 6.0, or another low-salt buffer, and add it to 100µL of the Fab or $F(ab')_2$ Stock Digestion Buffer.

Procedure for Digestion of Mouse IgG_1

Note: The enzyme-to-protein ratio, temperature, time, pH and salt concentration affect antibody digestion rates and location of cleavage.

1. Pack a column with 3-6mL of the Immobilized Ficin resin slurry (1-2mL of settled resin). For suspension digestion, add 3-6mL of the Immobilized Ficin resin slurry (1-2mL of settled resin) into a test tube or centrifuge tube.
2. Equilibrate the Immobilized Ficin with 20mL of Ficin Equilibration Buffer. Allow the buffer to flow through the column. For suspension digestion, centrifuge the tube for 1 minute at 1000 × *g* and remove 20mL of the buffer or use a Resin Separator (Product No. 69710) to remove the buffer.
3. Add the prepared mouse IgG_1 sample to the activated Immobilized Ficin. Add additional equilibration buffer to allow the sample to fully enter the resin bed when using the column format.
4. Incubate at 37°C at the time suggested in Table 1. For suspension digestion, begin mixing.

Table 1. Suggested digestion time.

Desired Fragment	Concentration (mg/mL)	Hours
Mouse IgG_1 Fab	0.5-10	3-5
Mouse IgG_1 $F(ab')_2$	0.5-3	20
Mouse IgG_1 $F(ab')_2$	5-10	40

5. If the fragments will be purified on a Protein A column, use a Protein A binding buffer to wash the digest from the Immobilized Ficin. Use a volume buffer 3-4 times the volume of digest mixture to promote subsequent binding of undigested IgG and Fc to Protein A.
6. To regenerate the Immobilized Ficin, wash with a neutral pH buffer containing 10mM EDTA. Store resin in a neutral pH buffer containing 10mM EDTA and 50% glycerol. Immobilized Ficin can be reused up to five times with approximately 85% retention of activity.

Separation of Fragments Using Protein A

Because Protein A does not bind to $F(ab')_2$ and Fab fragments Fab and $F(ab')_2$, Fc and undigested IgG can be removed using Protein A Agarose (e.g., Product No. 20333). Mouse IgG_1 and Fc fragments bind to immobilized Protein A and can be eluted using Mouse IgG_1 Elution Buffer (Product No. 21034). For storage, dialyze the Fab or $F(ab')_2$ fragments into phosphate-buffered saline or Tris-buffered saline using Thermo Scientific Slide-A-Lyzer Dialysis Cassettes (Product No. 66425) or other suitable product. Purity is typically > 98% $F(ab')_2$ and > 96% Fab after dialysis. Protein recoveries may vary from 35-58%, depending on the amount of starting antibody, the processing method(s), and the protein assays used.

General References

1. Mariani, M., *et al*. (1991). A new enzymatic method to obtain high-yield $F(ab')_2$ suitable for clinical use from mouse IgG_1. *Mol Immunol* **28:**69-77.
2. Milenic, D.E., *et al*. (1989). Comparison of methods for the generation of immunoreactive fragments of a monoclonal antibody (B72.3) reactive with human carcinomas. *J Immunol Methods* **120:**71-83.
3. Carrey, E.A. in Protein Structure: A Practical Approach (T.E. Creighton ed.) IRL Press, 1989, Oxford, p. 117.
4. Leiner, I.E. and Friedenson, B. (1970). Ficin. *Methods Enzymol* **19:**261-73.
5. Boguslawski, S.J., *et al.* (1989). Improved procedure for preparation of $F(ab')_2$ fragments of mouse IgGs by papain digestion. *J Immunol Meth* **120:**51-56.

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