Mouse IgG1 Fab and F(ab´)2 Micro Preparation Kit

BIOK2025-M

Mouse IgG1 Fab and F(ab')2 Micro Preparation Kit, contains sufficient reagents to generate and purify Mouse IgG1 Fab and F(ab´)2 fragments from ten 0.125mL samples containing 25-250µg of IgG

Kit Contents:

Immobilized Ficin	0.8mL*1
Microcentrifuge Tubes	1.5mL*10
Cysteine•HCl•H2 O	1g*1
Mouse IgG1 Digestion Buffer, pH 6.0	30ml*1
Protein A Columns	0.2mL*2
Protein A Binding Buffer	30ml*1
IgG Elution Buffer, pH 2.8	30ml*1
Desalting Columns	0.5ml*10
Microcentrifuge Tubes	2.0mL*30

Storage: Upon receipt store at 4-8°C. Product is shipped at ambient temperature

Introduction

The Mouse IgG1 Fab and F(ab´)2 Preparation Kit uses Immobilized Ficin to prepare fragments from mouse IgG1. Ficin generates F(ab´)2 fragments exclusively in the presence of 1-4mM cysteine; and Fab fragments are generated in the presence of 25mM cysteine (Figure 1). Fragment generation from other IgG species and isotypes might be possible by modifying the cysteine concentration and other digestion parameters.

Pepsin is commonly used for generating F(ab´)2 fragments because the pepsin cleavage site on human IgG contains a Leu 234, which is conserved in most species; however， mouse IgG1 lacks this residue and others, which possibly contributes to the restricted hinge region and resistance to pepsin cleaveage. Also, the low pH required for pepsin digestion can destroy or damage

antibodies. For comparison, mouse IgG1 monoclonal antibodies were digested with ficin, pepsin, bromelain and elastase. Ficin digestion produced high yields of F(ab´)2 fragments with the highest residual antigen-binding activity and immunoreactivity. Affinity constants of ficin-generated F(ab´)2 fragments were near those of intact antibody.

This kit contains the necessary components for Fab or F(ab´)2 generation of mouse IgG1 and subsequent purification. Immobilized Ficin enables immediate cessation of the digestion by simply removing the resin from the antibody digest solution. The included Columns allow easy manipulation of the resin and maximum Fab and F(ab´)2 recoveries. The prepacked immobilized Protein A column and optimized binding buffer binds the intact Fc fragments and undigested IgG, allowing for efficient Fab or F(ab´)2 fragments purification. The optimized cysteine concentration produces Fab or F(ab´)2 with maximum purity. This complete kit makes Fab and F(ab´)2 generation and purification simple, fast and effective.

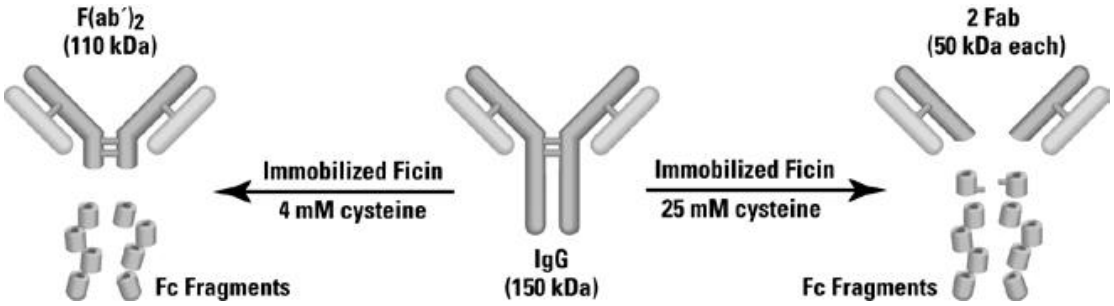

Figure 1. Using ficin with different concentrations of cysteine produces either Fab or F(ab´)2 fragments

Important Product Information

- These instructions are optimized for mouse IgG1. Fragmentation of other mouse IgG isotypes or IgG from other species might require optimization.
- The kit components and protocol are for 0.125mL samples containing 25-250µg IgG.
- Proper sample preparation is essential for successful fragment generation using this kit. If the IgG sample contains a carrier protein such as BSA, removing it before performing the

buffer exchange.

- Protein A Binding Buffer will precipitate in SDS-PAGE Loading Buffer. Dilute sample 1:5 or desalt or dialyze before loading onto a gel.

Additional Materials Required

- Incubator capable of maintaining 37°C
- Microcentrifuge capable of 5000 × *g*
- Variable speed centrifuge
- 15mL conical collection tubes
- End-over-end mixer or tabletop rocker

Material Preparation

Digestion Buffer

1) Fab generation: Dissolve 43.9mg cysteine•HCl in 10mL of the supplied Mouse IgG1 Digestion Buffer. After adding the cysteine•HCl the pH should be ~5.6.
2) F(ab´)2 generation: Dissolve 7mg cysteine•HCl in 10mL of the supplied Mouse IgG1 Digestion Buffer. After adding the cysteine•HCl the pH should be ~5.9
3) Note: Cysteine readily oxidizes to cystine; therefore, prepare this buffer on the same day of use.

Procedure for Fab or F(ab´)2 Generation and Purification

A. Immobilized Ficin Equilibration

1. Gently swirl the Immobilized Ficin vial to obtain an even suspension. Seat the spin-column frit with an inverted 200µL pipette tip.
2. Using a wide-bore or cut pipette tip, place 200µL into the 1.5mL Microcentrifuge Tubes. Centrifuge the column at 5000 × *g* for 1 minute and discard buffer.
3. Wash resin with 0.5mL of Digestion Buffer. Centrifuge column at 5000 × *g* for 1

minute and discard supernatant buffer.

B. IgG Sample Preparation

1. Place the Desalting column in a 2mL collection tube.
2. Centrifuge the column at 1500 × *g* for 1 minute to remove storage solution. Place a mark on the side of the column where the compacted resin is slanted upward. Place column in centrifuge with the mark facing outward in all subsequent centrifugation steps.

   Note: Resin will appear compacted after centrifugation.
3. Add 300µL of Digestion Buffer on top of the resin bed. Centrifuge at 1500 × *g* for 1 minute to remove buffer. Repeat this step three additional times, discarding buffer from the collection tube.
4. Place column in a new collection tube, and slowly apply 0.125mL of sample to the center of the compacted resin bed.
5. Replace cap and centrifuge at 1500 × g for 2 minutes to collect the sample. Discard the column after use.
6. If IgG sample is 0.2-2mg/mL (i.e., 25-250µg), no further preparation is necessary. If sample volume is less than 125µL, add Digestion Buffer to a final volume of 125µL.

C. Generation of Fragments

1. Add 125µL of the prepared IgG sample to the Microcentrifuge Tubes containing the equilibrated Immobilized Ficin.
2. Incubate digestion reaction 3-5 hours for generation of Fab fragments or 24-30 hours for generation of F(ab´)2 fragments with end-over-end mixer or tabletop rocker at 37°C. Maintain constant mixing of resin during incubation.
3. Centrifuge the microcentrifuge tube at 5000 × g. for 1 minute to separate digest from the Immobilized Ficin.
4. Wash resin with 125µL Protein A Binding Buffer. Place spin column into a 2.0mL microcentrifuge tube. Centrifuge column at 5000 × *g* for 1 minute. Repeat this step

for a total of three washes.

5. Add the wash fractions to the digested antibody from Step 3. Total sample volume should be 0.5mL. Discard used Immobilized Ficin.

   Note: To assess digestion completion, evaluate the digest and wash fraction via SDS-PAGE. Dilute digest 1:5 before adding to SDS-PAGE loading buffer. Because of the presence of cysteine, boiling samples in non-reducing SDS-PAGE loading buffer will reduce the sample. To avoid reducing the 50kDa Fab fragment or 110kDa F(ab´)2 fragment do not boil the samples. For best interpretation, desalt or dialyze samples before electrophoresis.

D. Fab and F(ab´)2 Purification

1. Equilibrate the Protein A Column, Protein A Binding Buffer and IgG Elution Buffer to room temperature. Set centrifuge to 1000 × *g*.
2. Place column in a 2mL collection tube and centrifuge for 1 minute to remove storage solution. Discard the flow-through.
3. Equilibrate column by adding 400µL of Protein A Binding Buffer and briefly mix. Centrifuge for 1 minute and discard the flow-through. Repeat this step once.
4. Apply 25-500µL of sample to column and tightly cap top. Resuspend the resin and sample by inversion. Incubate at room temperature with end-over-end mixing for 2 minutes.
5. Place column in a new 2mL collection tube and centrifuge for 1 minute. Save the flow-through as this fraction contains Fab and F(ab´)2 fragments.
6. For optimal recovery, wash column with 200µL of Protein A Binding Buffer. Centrifuge for 1 minute and collect the flow-through. Repeat and combine wash fractions with the Fab or F(ab´)2 fraction from Step 5.
7. Apply 400µL of IgG Elution Buffer to the Protein A Column and centrifuge for 1 minute. Repeat this step two times to obtain three fractions, which will contain undigested IgG and Fc fragments. To save the undigested IgG or Fc fragments, add 40µL of a

neutralization buffer (e.g., 1M phosphate or 1M Tris at pH 8-9) to each elution fraction.

8. Estimate protein concentration by measuring the absorbance at 280nm. Use an estimated extinction coefficient of 1.4.

E. Regeneration of the Immobilized Protein A Column

1. Add 400µL of IgG Elution Buffer and centrifuge for 1 minute. Discard flow-through and repeat once.
2. Add 400µL of PBS to the column, centrifuge for 1 minute and discard the flow-through. Repeat three times.
3. For storage, add 400µL of 0.02% sodium azide in PBS to the column. Replace top and bottom caps. Store column upright at 4°C. Columns can be regenerated at least 10 times without significant loss of binding capacity.

F. Troubleshooting

Problem	Possible Cause	Solution
Low amounts of Fab (50kDa) or F(ab´)2 (110kDa) produced as visualized by non- reducing SDS-PAGE	IgG sample was not prepared properly	Buffer exchange IgG into Digestion Buffer
	Sample loading buffer contains reducing reagent	Use SDS loading buffer that does not contain β-mercaptoethanol, DTT or TCEP
	Digested material contains cysteine	Desalt digest before SDS-PAGE
	Sample contains protein other than IgG (e.g., BSA)	Remove BSA with the Antibody Clean-up Kit
	Some mouse IgG1 clones may generate alternate fragments of different molecular weight[2]	Use the Fab Preparation Kit or F(ab´)2 Preparation Kit and dilute mouse IgG1 samples with Protein A Binding Buffer for purification

Fab or F(ab´)2 has low immunoreactivity	Sample digested for too long	Reduce digestion time; do not exceed 8 hours for Fab or 40 hours for F(ab´)2 or try using the F(ab´)2 or Fab Preparation Kit
Protein A flow-through contains Fab and F(ab´)2	Extended digestion times for F(ab´)2 production might result in the formation of Fab	Use recommended cysteine concentrations and digestion times