MAK-0003

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Name	**F(ab')2 Preparation Kit**
Size	10 samples for 250ug-4mg IgG
Store	4–8°C.
Application	Mabioway F(ab')2 Preparation Kit uses immobilized pepsin protease to selectively digest whole human and other IgG antibodies to make F(ab')2 fragments that retain antigen binding activity.
Features	• Enzyme-free digestion products—Immobilized Pepsin (beaded agarose resin) provides for control of the digestion reaction and complete removal of resulting antibody fragments from the proteolytic enzyme • Suitable for human and other species of IgG—the kit procedure is optimized for human, rabbit and mouse IgG. Pepsin-based digestion is effective for other species and subclasses of IgG, although the purification step requires that the antibody effectively binds to Protein A. (Note: for best results with mouse IgG1, use Part No. MAK-0006) • Complete—kits include all reagents needed to prepare and purify antibody fragments • Fast— format greatly reduces sample processing time • Flexible—protocols are included for multiple species and IgG subclasses, as well as sample size and concentration. • Efficient—enhanced yield and sample purity
Product description	These F(ab')2 Preparation Kits are suitable for human, rabbit, mouse and other species and subclasses of IgG except mouse IgG1. The antibody digestion

reaction with pepsin agarose is performed in convenient disposable columns that allow efficient removal of the immobilized protease and maximum recovery of the IgG fragments. Also included in the kits are Protein A Columns and buffers to efficiently purify the resulting fragments. Protein A binds the Fc fragments and undigested IgG, allowing the pure F(ab')2 fragments to be recovered in the flow-through fraction. The kits also include Desalting Columns for preparing the IgG sample quickly without dilution instead of utilizing time-consuming dialysis steps.
The kits use pepsin, a nonspecific endopeptidase, to enzymatically digest the Fc portion of whole IgG to yield the fragment known as F(ab')2. This fragment is composed of a pair of Fab' units connected by two disulfide bonds. Because the pepsin protease is supplied in immobilized form as beaded agarose resin, the digestion reaction is easily stopped by removing the resin from the IgG solution; the result is digest products that are enzyme-free.

Contents

10 antibody samples, each containing 0.25 to 4 mg IgG
• Immobilized Pepsin Agarose, 1.25 mL
• F(ab')2 Digestion Buffer, 100 mL
• Protein A Column, 1 mL, 1 column
• PBS Packs (each makes 1000 mL), 1 pack
• IgG Elution Buffer, 100 mL
• Desalt Columns, 2 mL, 10 columns
• Microcentrifuge Tubes, 2 mL, 30 tubes

Product Information

• Proper sample preparation is essential for successful fragment generation using this kit. If the IgG sample contains a carrier protein such as BSA, use the Antibody Clean-up Kit (Product No. MAK-0007) to remove it before performing the buffer exchange (Section B).
• For best results, use rabbit, human or mouse IgG. Fragmentation of IgG from other species may require optimization. For purification, the IgG species must be able to bind to Protein A . For best results with mouse IgG1, use the IgG1 Fab and F(ab´)2 Preparation Kit (Product No. MAK-0006).
• The kit components and protocol are for 0.5mL samples containing 250µg-4mg of IgG per sample. For 25-250µg samples use the F(ab´)2 Micro Preparation Kit (Product No. MAK-0004).

Additional Materials

• Incubator capable of maintaining 37°C
• Microcentrifuge capable of 5,000 × g
• Variable speed centrifuge
• 15mL conical collection tubes
• End-over-end mixer or tabletop rocker

Material Preparation

Phosphate-buffered Saline (PBS): Dissolve contents of a package in 1000mL of ultrapure water. For long-term storage, add 0.05% sodium azide and store at 4°C.

Procedure

A. Immobilized Pepsin Equilibration

1. Gently swirl the Immobilized Pepsin vial to obtain an even suspension. Seat the spin column frit with an inverted 200µL pipette tip.
2. Tplace 150µL of the 50% slurry (i.e., 75µL of settled resin) into the 1.5mL Microcentrifuge Tubes at 5000 × g for 1 minute and discard buffer.
3. Wash resin with 500µL of Digestion Buffer. Centrifuge column at 5000 × g for 1 minute and discard buffer.

B. IgG Sample Preparation

1. Desalting Column and loosen cap. Place column in a 15mL collection tube.
2. Centrifuge 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 1000µL of Digestion Buffer to column. 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, apply 500µL of sample to the center of the compacted resin bed.
5. centrifuge at 1500 × g for 2 minutes to collect the sample. Discard the column after use.
6. If IgG sample is 0.5-8mg/mL (i.e., 250µg to 4mg), no further preparation is necessary. If sample volume is less than 500µL, add Digestion Buffer to a final volume of 500µL.

C. Fragment Generation

1. Add 500µL of the prepared IgG sample to the column containing the equilibrated Immobilized Pepsin (Section A). Briefly vortex to mix.
2. I Incubate digestion reaction for the appropriate time (see the Appendix) with an end-over-end mixer or a tabletop rocker at 37°C. Maintain constant mixing of resin during incubation.
3. Centrifuge column at 5000 × g for 1 minute to separate digest from the Immobilized Pepsin.
4. Wash resin with 500µL of PBS. Place column into a new tube and centrifuge at 5000 × g for 1 minute. Repeat this step once.
5. Add both wash fractions to the digested antibody. Total sample volume should be 1.5mL. Discard the Immobilized Pepsin.

Note: For best results, evaluate the digest and wash fraction via SDS-PAGE to assess digestion completion. Protein A purification is only required to remove undigested IgG. F(ab´)2 and degraded Fc do not bind to Protein A . The resulting F(ab´)2 in non-reducing SDS-PAGE derived from human and mouse IgG will migrate with an apparent molecular weight of ~110kDa. Rabbit F(ab´)2 will migrate with a lower apparent molecular weight of ~88kDa.

D. F(ab´)2 Purification

1. Equilibrate the Protein A Column, PBS and IgG Elution Buffer to room temperature. Set centrifuge to 1000 × g.
2. Place column in a collection tube and centrifuge for 1 minute to remove storage solution (contains 0.02% sodium azide). Discard the flow-through.
3. To equilibrate column, add 2mL of PBS and briefly mix. Centrifuge for 1 minute and discard the flow-through. Repeat this step once.
4. Apply the sample to column and cap the top tightly. Resuspend the resin and sample by inversion. Incubate at room temperature with end-over-end mixing for 10 minutes.
5. Place column in a new collection tube and centrifuge for 1 minute. Save the flow-through as this fraction contains F(ab´)2 and Fc fragments that are too small to bind to Protein A .
6. For optimal recovery, wash column with 1mL of PBS. Centrifuge for 1 minute and collect flow-through. Repeat and combine wash fractions with the F(ab´)2 fraction from Step 5.
7. Measure protein concentration using the BCA Protein A ssay or by measuring the absorbance at 280nm. Use an estimated extinction coefficient of 1.4. Assuming complete IgG digestion, F(ab´)2 yields may vary from 50 to 70%, depending on the amount of starting antibody and the Protein A ssays used.
8. If desired, perform dialysis (50K MWCO), gel filtration or ion-exchange chromatography to remove the Fc fragments that are too small to bind to Protein A.
E. Regeneration of the Immobilized Protein A Column
1. Apply 1mL of IgG Elution Buffer to the Protein A Column. Centrifuge for 1 minute. Repeat this step two times to obtain three fractions, which will contain undigested IgG. To save the undigested IgG, add 100μL of a neutralization buffer (e.g., 1M phosphate or 1M Tris at pH 8-9) to each elution fraction.
2. Add 1mL of IgG Elution Buffer to the column and centrifuge for 1 minute. Discard flow-through and repeat.
3. Add 1mL of PBS to the column and centrifuge for 1 minute. Discard flow-through and repeat two times.
4. For storage, add 1mL of 0.02% sodium azide in PBS to 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.

Trouble shooting

Problem 1: Low amounts of F(ab´)2 produced as determined by non-reducing SDS-PAGE
1)IgG sample was not in Digestion Buffer.
Dialyze or buffer exchange IgG into Digestion Buffer, or decrease the Digestion Buffer pH to 3-4.3 [note that decreasing the pH might increase the F(ab´)2 amount produced but can reduce its immunoreactivity].
2)Sample loading buffer contains reducing reagent.
Use SDS loading buffer that does not contain βmercaptoethanol, DTT or TCEP.
3)Resin was not equilibrated in Digestion Buffer before adding IgG.
Wash resin with 0.5mL of Digestion Buffer before adding IgG sample.
4)Sample was goat or mouse IgG1.
Reduce IgG concentration and increase digestion time to 8 hours.

5)Some mouse IgG1 were resistant to pepsin cleavage
Use the IgG1 Fab and F(ab´)2 Preparation Kit (Product No. 44980 or 44680).
6)Sample contained protein other than IgG (e.g., BSA), which can increase digestion time
Remove BSA with the Antibody Clean-up Kit (Product No. 44600).
Problem 2: F(ab´)2 has low immunoreactivity
1)Sample digested for too long.
Reduce digestion time; do not exceed 8 hours.
2)The low pH of Digestion Buffer decreased F(ab´)2 activity.
Use the IgG1 Fab and F(ab´)2 Preparation Kit.
Problem 3: Low F(ab´)2 recovery
1)Incomplete washing of the pepsin resin.
Two 500µL washes of PBS are required for maximum recovery.
Problem 4: A portion of undigested IgG does not bind to Protein A
1)Sample was goat or mouse IgG1.
a. Goat IgG binds weakly to Protein A , so try an alternative purification method such as ionexchange.
b. Dilute sample in Protein A Binding Buffer before adding to the Protein A Column.

Digestion Times

This kit is for digesting 0.5mL of IgG at 0.5-8mg/mL from rabbit, human or mouse. Digestion effectiveness will vary depending on antibody preparation and source (rate and completeness of digestion: rabbit > human > mouse ≥ goat). The times listed in Table 1 result in > 90% digestion of IgG. Data was generated using serum purified by immobilized Protein A or G affinity chromatography. Digestion over 8 hours is not recommended.

Gel Interpretation