MAK-0001

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

PRODUCT INFORMATION

Name	**Fab Micro Preparation Kit**
Size	10 samples for 25-250ug IgG
Store	4–8°C.
Application	Mabioway Fab Micro Preparation Kit uses immobilized papain protease to digest human or mouse IgG antibodies to make separate Fab and Fc fragments and subsequently to purify the Fab using Protein A agarose.
Features	• High capacity—use the micro kit for 25 to 250 µg IgG. • Enzyme-free digestion products—Immobilized Papain (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, mouse and rabbit IgG. Papain-based digestion is effective for certain other species and subclasses of IgG (e.g., goat and pig), although the purification step requires that the antibody effectively binds to Protein A . (Note: for best results with mouse IgG1, use Part No. MAK-0005 OR MAK-0006.) • Provides ready-to-use Fab—digestion and final recovery of purified Fab fragments occurs in neutral pH sodium phosphate buffer, suitable for storage or immediate use in typical applications • 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 Fab Preparation Kits are suitable for human, rabbit, mouse and other species and subclasses of IgG. The papain antibody digestion reaction 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 Fab fragments to be recovered in the flow-through fraction. The kits also include Mabioway Desalting Columns for preparing the IgG sample quickly without dilution instead of utilizing time-consuming dialysis steps.

The kits use papain, a nonspecific thiol-endopeptidase, to enzymatically cleave whole IgG just above the hinge region to create two separate Fab fragments and one Fc fragment per antibody molecule. Because the papain 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 25 to 250 µg IgG
- Immobilized Papain Agarose, 0.5 mL
- Cysteine-HCl, 0.5 g
- Fab Digestion Buffer, 30 mL
- Protein A Columns, 0.2 mL, 2 columns
- PBS 1 pack for 1L
- IgG Elution Buffer, 30 mL
- Desalt Columns, 0.5 mL, 10 columns
- Microcentrifuge Tubes, 2 mL, 10 tubes

Product Information

- These instructions are optimized for rabbit, human and mouse IgG. Fragmentation of IgG from other species might require optimization. For purification, the IgG species must be able to bind to Protein A . For mouse IgG1, use the IgG1 Fab and F(ab')2 Micro Preparation Kit (Product No. MAK-0005).
- Digestion effectiveness will vary depending on antibody preparation and source (rate and completeness of digestion: mouse > rabbit > human). Digestion times indicated in the protocol produce > 90% digestion for mouse and rabbit IgG and > 80% digestion for human IgG, using serum purified by Protein A or G affinity chromatography.
- The kit components and protocol are for 0.125mL samples containing 25-250µg of IgG. For 250µg-4mg samples use the Fab Preparation Kit (Product No.MAK-0002).
- 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).

Additional Materials

- Incubator capable of maintaining 37°C
- Microcentrifuge capable of 5000 × g
- Variable speed centrifuge
- 1.5mL microcentrifuge tubes
- End-over-end mixer or tabletop rocker

Material Preparation

- Digestion Buffer: Dissolve 14mg cysteine•HCl in 4mL of the supplied Fab Digestion Buffer (pH 10). After adding the cysteine•HCl the pH should be ~7.0. Note: Cysteine readily oxidizes to cystine; therefore, prepare this buffer on the same day of use.
- 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 Papain Equilibration

1. Gently swirl the Immobilized Papain vial to obtain an even suspension.
2. Tplace 50µL of the 50% slurry (i.e., 25µL of settled resin) into the 1.5mL Microcentrifuge Tubes at 5000 × g for 1 minute and discard buffer.
3. Wash resin with 130µL of Digestion Buffer. Centrifuge column at 5000 × g for 1 minute and discard buffer.

B. IgG Sample Preparation

1. Desalting Column, 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.
2. Add 300µL 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.
3. Place column in a new Microcentrifuge Tubes, slowly apply 125µL of sample to the center of the compacted resin bed.
4. Centrifuge at 1500 × g for 2 minutes to collect the sample. Discard the column after use.
5. 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. Fragment Generation

1. Add 125µL of the prepared IgG sample to the column tube containing the equilibrated Immobilized Papain. Briefly vortex the Tube.
2. Incubate the digestion reaction 5-6 hours 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 Papain.
4. Wash resin with 130µL of PBS. centrifuge at 5000 × g for 1 minute.
5. Add the wash fraction to the digested antibody from Step 3. Total sample volume should be 255µL. Discard the used Immobilized Papain.
Note: To assess digestion completion, evaluate the digest and wash fraction via SDS-PAGE. The separated digest and wash fraction contains cysteine. Boiling samples in non-reducing SDS-PAGE loading buffer will reduce the sample. To avoid reducing the 50kDa Fab fragment on SDS-PAGE, do not boil the samples.
D. Fab Purification
1. Protein A Plus Column, PBS 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 300µL of PBS and briefly mix. Centrifuge the column for 1 minute and discard the flowthrough. Repeat this step once.
4. Apply 25-500µL of sample to column. 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 2mL collection tube and centrifuge for 1 minute. Save the flow-through as this fraction contains Fab fragments.
6. For optimal recovery, wash column with 200µL of PBS. Centrifuge for 1 minute and collect the flow-through. Repeat and combine wash fractions with the Fab fraction from Step 5.
7. Apply 300µL 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. Measure protein concentration by absorbance at 280nm. Use an estimated extinction coefficient of 1.4. Assuming complete IgG digestion, Fab yields may vary from 50 to 65%, depending on the amount of starting antibody and the Protein A ssays used. Protein concentration may also be measured using the BCA Protein A ssay; however the sample must contain less than 2.5mM cysteine. The undiluted digest and Protein A fraction contains approximately 5mM cysteine.
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.

Trouble shooting

Problem 1: Low amounts of Fab (50kDa) produced as visualized by nonreducing SDS-PAGE

1)The IgG sample was not properly prepared:
Dialyze or buffer-exchange IgG into the Digestion Buffer
2)Cysteine in the Digestion Buffer oxidized to cystine:
Prepare Digestion Buffer with cysteine on the same day of usage
3)Sample loading buffer contains reducing reagent:
Use SDS loading buffer that does not contain β-mercaptoethanol, DTT or TCEP
4)Digested material contains cysteine
Wash resin with 130µL of Digestion Buffer before adding IgG sample
5)Sample contains protein other than IgG (e.g., BSA), which can increase digestion time:
Purify the antibody sample with the Antibody Clean-up Kit
Problem 2: Fab has low immunoreactivity
1)Sample was digested for too long
Reduce digestion time and do not exceed 20 hours or try using the F(ab´)2 Micro Preparation Kit
Problem 3: A portion of undigested IgG or Fc does not bind to Protein A
1)Sample is goat IgG
Try an alternative purification method such as ionexchange chromatography
2)Sample is mouse IgG1
Dilute mouse IgG1 sample in Protein A Binding Buffer before adding to the Protein A Column

Digestion Times

Gel Interpretation