Fab Preparation Kit

Cat No: BIOK2024

Size: 10 Antibody Samples

PRODUCT DESCRIPTION

These Fab preparation kits are suitable for humans, rabbits, mice, and other species and the IgG subclass. The papain antibody digestion reaction is carried out in a convenient disposable centrifugal column, which can efficiently remove the immobilized protease and greatly recover IgG fragments. The kit also contains a NAb protein A centrifuge column and a buffer solution that can efficiently purify the resulting fragment. Protein A binds to Fc fragments and non digested IgG antibodies, and the purified Fab fragments can be recovered from the column flow fraction. The kit also includes a desalinated centrifuge column for rapid preparation of IgG samples without the need for dilution and time-consuming dialysis steps.

This kit uses papain (a non specific thiol endopeptidase) to enzymatically cleave the entire IgG from directly above the hinge region, producing two independent Fab fragments and one Fc fragment per antibody molecule. As papain is provided in the form of immobilized bead like agarose resin, the enzymatic digestion reaction can be easily stopped by removing the resin from the IgG solution; The result is an enzyme free enzymatic digestion product.

PROVIDED MATERIALS

Available for: 10 antibody samples, each containing 25 to 250ug of IgG

- Papain enzyme agarose, 0.5 mL
- Cysteine hydrochloride, 0.5g
- Fab digestion buffer, 55 mL
- Protein A column, 0.2 mL, 2 column
- PBS packages (each can prepare 500 mL), 2 packages
- IgG elution buffer, 50 mL
- Spin Columns, 0.5mL, 10 columns

- Microcentrifuge tube, 2 mL, 30 tubes

Storage method: Store at 4-8 ℃, do not freeze.

Important Product Information

- These instructions are optimized for rabbit, human and mouse IgG (25μg-250ug per sample). Fragmentation of IgG from other species might require optimization. For purification, the IgG species must be able to bind to Protein A.
- The kit components and protocol are for 0.25mL samples containing 25-250μg of IgG.
- 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 to remove it before performing the buffer exchange.

Additional Materials Required

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

Material Preparation

- Digestion Buffer: Dissolve 15mg 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 500mL of ultrapure water. For long-term storage, add 0.05% sodium azide and store at 4° C.

Procedure for Fab Generation and Purification

A、Immobilized Papain Equilibration

1. Gently swirl the Immobilized Papain 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 0.25mL of the 50% slurry (i.e., 0.125mL of settled resin) into the 0.8mL spin column. Centrifuge the column at 5000g for 1 minute and discard buffer.

3. Wash resin with 0.5mL of Digestion Buffer. Centrifuge column at 5000g for 1 minute and discard buffer.

B、IgG Sample Preparation

1. Place Desalting Column in a 2mL collection tube.

2. Centrifuge column at 1000g for 2 minutes 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 1mL of Digestion Buffer to column. Centrifuge at 1000g for 2 minutes to remove buffer. Repeat this step three additional times, discarding buffer from the collection tube.

4. Place column in a new collection tube, open cap and slowly apply 0.25mL sample to the center of the compacted resin bed.

5. Replace cap and centrifuge at 1000g for 2 minutes to **collect the sample**. Discard the column after use.

6. If IgG sample is 0.2-2mg/mL (i.e., 25µg to 250ug), no further preparation is necessary. If sample volume is less than 0.25mL, add Digestion Buffer to a final volume of 0.25mL.

C、Generation of Fragments

1. Add 0.25mL of the prepared IgG sample to the spin column tube containing the equilibrated Immobilized Papain.

2. Incubate the digestion reaction for the appropriate time (see Appendix A) with an end-over-end mixer or a tabletop rocker at 37° C. Maintain constant mixing of resin during incubation.

3. Remove bottom cap and place spin column into a microcentrifuge tube. Centrifuge column at 5000g for 1 minute to separate digest from the Immobilized Papain.

4. Wash resin with 0.25mL PBS. Place spin column into a microcentrifuge tube. Centrifuge column at 5000g for 1 minute.

5. Add the wash fraction to the digested antibody from Step 3 Total sample volume should be 0.5mL. 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. See representative gel in Appendix B.

D、Fab Purification

1. Protein A Column, PBS and IgG Elution Buffer to room temperature. Set centrifuge to 1000g.

2. Place Protein A Column in a 2mL collection tube and centrifuge for 1 minute to remove storage solution (contains 0.02% sodium azide). Discard the flow-through.

3. Equilibrate column by adding 0.4mL of PBS, centrifuge for 1 minute and discard the flow-through. Repeat this step once.

4. Apply 25-500ul sample to column and tightly cap top. 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 200ul 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 0.4mL 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 of the elution fractions.

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 assays used. Protein concentration may also be measured using the BCA Protein Assay; 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 0.4mL of IgG Elution Buffer and centrifuge for 1 minute. Repeat and discard flow-through.

2. Add 0.4mL of PBS to the column, centrifuge for 1 minute and discard the flow-through. Repeat three times.

3. For storage, add 0.4mL 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.

Troubleshooting

Problem	Possible Cause	Solution
Low amounts of Fab (50kDa) produced as visualized by nonreducing SDS-PAGE	The IgG sample was not properly prepared	Dialyze or buffer-exchange IgG into the Digestion Buffer
	Cysteine in the Digestion Buffer oxidized to cystine	Prepare Digestion Buffer with cysteine on the same day of usage
	Sample loading buffer contains reducing reagent	Use SDS loading buffer that does not contain β-mercaptoethanol, DTT or TCEP
	Digested material contains cysteine	Desalt before SDS-PAGE
	Resin not equilibrated in Digestion Buffer	Wash resin with 0.5mL of Digestion Buffer before adding IgG sample
	Sample contains protein other than IgG (e.g., BSA), which can increase digestion time	Purify the antibody sample with the Pierce Antibody Clean-up Kit
Fab has low	Sample was digested for too	Reduce digestion time and do not

immunoreactivity	long	exceed 20 hours or try using the F(ab´)2 Preparation Kit
A portion of undigested IgG or Fc does not bind to Protein A	Sample is goat IgG	Try an alternative purification method such as ion-exchange chromatography
	Sample is mouse IgG1	Dilute mouse IgG1 sample in Protein A Binding Buffer before adding to the Protein A Plus Spin Column

Appendix

A. Protein Gel Interpretation

The Fab and Fc analyzed by non-reducing and non-boiled SDS-PAGE typically migrate with an apparent molecular weight of 45-50kDa, depending on the antibody species. In reducing SDS-PAGE, Fab fragments migrate near 25kDa, and Fc fragments migrate at 28-30kDa. The presence of the Fc at 28-30kDa confirms digestion of IgG. Boiling the IgG digest before gel loading will result in a reduced sample, because of the cysteine present. Also, an additional band might be present in reduced SDS-PAGE, which is likely the undigested IgG heavy chain (50kDa).

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