F(ab')2 Preparation Kit

Cat No: BIOK2023

Size: 10 Antibody Samples

PRODUCT DESCRIPTION

These F (ab ') 2 preparation kits are suitable for IgG in humans, rabbits, mice, and other species and subclasses, with the exception of mouse IgG1. Using pepsin agarose in a convenient disposable centrifuge column for antibody digestion can efficiently remove immobilized proteases and recover IgG fragments as much as possible. The kit also contains a Thermo Scientific NAb protein A centrifuge column and buffer solution, which can efficiently purify the resulting fragments. Protein A can bind to Fc fragments and non digested IgG to recover pure F (ab ') 2 fragments from the flowthrough fraction. The kit also includes a Zeba desalting centrifuge column for rapid preparation of IgG samples without the need for dilution and time-consuming dialysis steps.

This kit contains the necessary components for F(ab´)2 generation and subsequent purification. Immobilized Pepsin is advantageous because the digestion can be immediately stopped by simply removing the resin from the antibody digest solution. The included Spin Columns allow easy manipulation of the resin and maximum F(ab´)2 recovery. The prepacked, immobilized Protein A Plus Spin Column binds the large Fc fragments and undigested IgG, allowing the F(ab´)2 fragments to pass through the column for efficient purification. This complete kit makes F(ab´)2 generation and purification simple, fast and effective.

PROVIDED MATERIALS

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

- Papain enzyme agarose, 0.5 mL
- F(ab')2 digestion buffer, 55 mL
- NAb Protein A Plus centrifuge column, 0.2 mL, 2 column
- PBS packages (each can prepare 500 mL), 2 packages

- IgG elution buffer, 50 mL
- Zeba desalting centrifuge column, 7K MWCO, 0.5 mL, 10 columns
- Centrifugal column, 0.8 mL, 10 columns
- Microcentrifuge tube, 2 mL, 30 tubes

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

Important Product Information

- The kit components and protocol is for 125µL samples containing 25-250µg of IgG per sample. For 250µg-4mg samples use the F(ab´)2 Preparation Kit.
- 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 Thermo Scientific Pierce Antibody Clean-up Kit to remove it before performing the buffer exchange.
- 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 IgG1 Fab and F(ab')2 Preparation Kit.
- Digestion effectiveness will vary depending on antibody preparation and source (rate and completeness of digestion:rabbit > human > mouse ≥ goat). Digestion times in the protocol result in > 90% digestion of IgG using serum purified by Protein A or G affinity chromatography. Digestion over 3 hours is not recommended.

Additional Materials Required

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

Material Preparation

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 Generating and Purifying F(ab')2 Fragments

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. Twist off the bottom tab from a 0.8mL spin column and place into a 2mL microcentrifuge tube. Using a wide-bore or cut pipette tip, place 65uL of the 50% slurry (i.e., 32.5uL of settled resin) into the 0.8mL spin column. Centrifuge the column at 5000 × g for 1 minute and discard buffer.

3. Wash resin with 130uL of Digestion Buffer. Centrifuge column at 5000 × g for 1 minute and discard buffer. Cap bottom of spin column with supplied rubber cap.

B、IgG Sample Preparation

1. Twist off the bottom closure of a Zeba Spin Desalting Column and loosen red cap. Place column in a 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 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.

4. Place column in a new collection tube, remove cap and slowly apply 125µL 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、Fragment Generation

1. Add125uL of the prepared IgG sample to the spin column tube containing the equilibrated Immobilized Papain. Place top cap and bottom plug on spin column.

2. Incubate digestion reaction for 2 hours for rabbit or human IgG or 3 hours for mouse IgG with an end-over-end mixer or tabletop rocker at 37° C. Maintain constant mixing of resin during incubation.

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

4. Wash resin with 130µL 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 385µL. 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 NAb Protein A Plus Spin Column, PBS and IgG Elution Buffer to room temperature. Set centrifuge to 1000 × g.

2. Snap off bottom closure and loosen top yellow cap on the Protein A Column. 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 400µL of PBS and briefly mix. Centrifuge for 1 minute and discard the flow-through. Repeat this step once.

4. Cap bottom of column with the included rubber cap. Apply 25-500µL 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. Loosen top cap and remove bottom cap. 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 200µL 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 Thermo Scientific BCA Protein Assay 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 assays 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 400µL of IgG Elution Buffer to the NAb Protein A Plus Spin 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 40µL of a neutralization buffer (e.g., 1M phosphate or 1M Tris at pH 8-9) to each elution fraction.

2. Add 400µL of IgG Elution Buffer to the column and centrifuge for 1 minute. Discard flow-through and repeat.

3. Add 400µL of PBS to the column and centrifuge for 1 minute. Discard flow-through and repeat two times.

4. For storage, add 400µL 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 F(ab´)2 produced as determined by non-reducing SDS-PAGE	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]
	Sample loading buffer contains reducing reagent	Use SDS loading buffer that does not contain β mercaptoethanol, DTT or TCEP
	Resin was not equilibrated in Digestion Buffer before adding IgG	Wash resin with 0.5mL of Digestion Buffer before adding IgG sample
	Sample is goat or mouse IgG1	Reduce IgG concentration and increase digestion time to 8 hours
	Some mouse IgG1 are resistant to pepsin cleavage	Use the Pierce IgG1 Fab and F(ab´)2 Preparation Kit
	Sample contains protein other than IgG (e.g., BSA), which can increase digestion time	Remove BSA with the Pierce Antibody Clean-up Kit
F(ab´)2 has low immunoreactivity	Sample digested for too long	Reduce digestion time; do not exceed 8 hours
	The low pH of Digestion Buffer	Use the Pierce IgG1 Fab and F(ab

	decreased F(ab´)2 activity	´)2 Preparation Kit
Low F(ab´)2 recovery	Incomplete washing of the pepsin resin	Two 130µL washes of PBS are required for maximum recovery
A portion of undigested IgG does not bind to Protein A	Sample is goat or mouse IgG1	Goat IgG binds weakly to Protein A, so try an alternative purification method such as ion-exchange
		Dilute sample in Pierce Protein A Binding Buffer before adding to the Protein A Column

Contact Us

QQ:499854788

3494243873

WeChat: 13681256816

15511114213

Email: info@biotyscience.com

Tel: 400-669-8850

13681256816