

Pierce[®] F(ab')₂ Preparation Kit

44988

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Number	Description
44988	<p>Pierce F(ab')₂ Preparation Kit, contains sufficient reagents to generate and purify F(ab')₂ fragments from up to ten 0.5 ml samples containing 0.25-4 mg of IgG</p> <p>Kit Contents:</p> <p>Immobilized Pepsin, 1.25 ml settled resin, contains 2-3 mg (> 6,000 units) of pepsin per milliliter of settled resin; support is 6% crosslinked beaded agarose supplied as a 50% slurry in 50% glycerol, 0.1 M sodium acetate, pH 4.4; 0.05% sodium azide</p> <p>NAb™ Protein A Spin Column, 1 ml, 1 each, binding capacity: ≥ 34 mg of human IgG per column</p> <p>BupH™ Phosphate Buffered Saline, 2 packs, makes 1 L of 0.1 M sodium phosphate, 0.15 M sodium chloride; pH 7.2</p> <p>IgG Elution Buffer, 120 ml, pH 2.8, contains primary amine</p> <p>Spin Columns, 10 each, 0.8 ml columns with caps and bottom plugs</p> <p>Microcentrifuge Tubes, 30 each, 2.0 ml collection tubes</p> <p>Digestion Buffer, 120 ml, 20 mM sodium acetate, pH 4.4; 0.05% sodium azide</p> <p>Zeba™ Desalt Spin Columns, 2 ml, 10 each, for 200-700 µl samples</p>

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

Introduction

The Thermo Scientific Pierce F(ab')₂ Preparation Kit enables efficient generation of F(ab')₂ from IgG. This kit uses Immobilized Pepsin, a nonspecific endopeptidase that is active only at acid pH and irreversibly denatured at neutral or alkaline pH. Pepsin digestion typically produces a F(ab')₂ fragment (~110 kDa by SDS-PAGE under non-reducing conditions) and numerous small peptides of the Fc portion (Figure 1). The resulting F(ab')₂ fragment is composed of a pair of Fab' units connected by two disulfide bonds. The Fc fragment is extensively degraded and can be separated from F(ab')₂ by dialysis, gel filtration or ion exchange chromatography.

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 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.

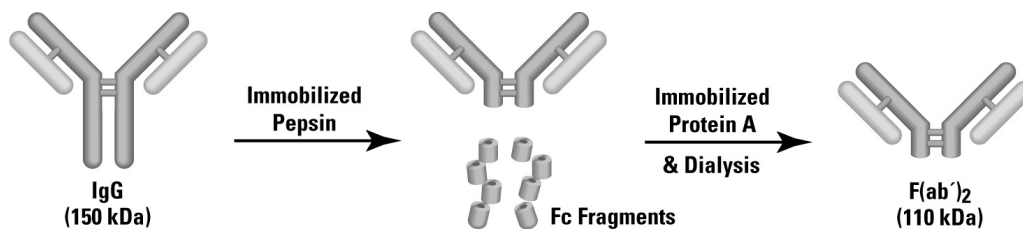


Figure 1. Schematic for preparing F(ab')₂ using Immobilized Pepsin and Protein A.

Important 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 Pierce Antibody Clean-up Kit (Product No. 44600) 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 Pierce IgG₁ Fab and F(ab')₂ Preparation Kit (Product No. 44980).
- The kit components and protocol are for 0.5 ml samples containing 250 µg-4 mg of IgG per sample. For 25-250 µg samples use the Pierce F(ab')₂ Micro Preparation Kit (Product No. 44688).

Additional Materials Required

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

Material Preparation

Phosphate-buffered Saline (PBS): Dissolve contents of a package in 500 ml 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 Pepsin Equilibration

1. Gently swirl the Immobilized Pepsin vial to obtain an even suspension. Seat the spin column frit with an inverted 200 µl pipette tip.
2. Using a wide-bore or cut pipette tip, place 0.25 ml of the 50% slurry (i.e., 0.125 ml of settled resin) into a 0.8 ml spin column. Twist off the bottom tab of the column, cap column and place into 2.0 ml microcentrifuge tube. Centrifuge column at 5,000 × g for 1 minute and discard buffer.
3. Wash resin with 0.5 ml Digestion Buffer. Centrifuge column at 5,000 × g for 1 minute and discard buffer. Cap bottom of spin column with included rubber cap.

B. IgG Sample Preparation

1. Twist off the bottom closure of a Zeba Desalt Spin Column and loosen cap. Place column in a 15 ml collection tube.
2. Centrifuge column at 1,000 × g 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 1 ml of Digestion Buffer to column. Centrifuge at 1,000 × g 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, remove cap and slowly apply 0.5 ml sample to the center of the compacted resin bed.
5. Replace cap and centrifuge at 1,000 × g for 2 minutes to collect the sample. Discard the column after use.
6. If IgG sample is 0.5-8 mg/ml (i.e., 250 µg to 4 mg), no further preparation is necessary. If sample volume is less than 0.5 ml, add Digestion Buffer to a final volume of 0.5 ml.

C. Fragment Generation

1. Add 0.5 ml of the prepared IgG sample to the spin column containing the equilibrated Immobilized Pepsin (Section A). Place top cap and bottom plug on the spin column.
2. 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. Remove bottom cap and place column into a 2.0 ml microcentrifuge tube. Centrifuge column at $5,000 \times g$ for 1 minute to separate digest from the Immobilized Pepsin.
4. Wash resin with 0.5 ml of PBS. Place column into a tube and centrifuge at $5,000 \times g$ for 1 minute. Repeat this step once.
5. Add both wash fractions to the digested antibody. Total sample volume should be 1.5 ml. 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 ~110 kDa. Rabbit F(ab')₂ will migrate with a lower apparent molecular weight of ~88 kDa.

D. F(ab')₂ Purification

1. Equilibrate the NAb Protein A Column, PBS and IgG Elution Buffer to room temperature. Set centrifuge to $1,000 \times g$.
2. Loosen top cap on the Protein A Column and snap off bottom closure. Place column in a 15 ml 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 2 ml of PBS. Centrifuge for 1 minute and discard the flow-through. Repeat this step once.
4. Cap bottom of column with the included rubber cap. Apply 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 15 ml collection tube and centrifuge for 1 minute. Save the flow-through as this fraction contains F(ab')₂ and Fc fragments.
6. For optimal recovery, wash column with 1 ml 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 Pierce BCA Protein Assay or by measuring the absorbance at 280 nm. 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.

E. Regeneration of the Immobilized Protein A Column

1. Apply 1 ml 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. To save the undigested IgG, add 100 µl of a neutralization buffer (e.g., 1 M phosphate or 1 M Tris at pH 8-9) to each of the elution fractions.
2. Add 3 ml of IgG Elution Buffer to the column and centrifuge for 1 minute. Discard flow-through and repeat.
3. Add 3 ml of PBS to the column and centrifuge for 1 minute.
4. For storage, add 3 ml 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 0.5 ml of Digestion Buffer before adding IgG sample
	Sample is goat or mouse IgG ₁	Reduce IgG concentration and increase digestion time to 8 hours
	Some mouse IgG ₁ are resistant to pepsin cleavage ¹	Use the Pierce IgG ₁ Fab and F(ab') ₂ Preparation kit (Product No. 44980 or 44680)
	Sample contains protein other than IgG (e.g., BSA), which can increase digestion time	Remove BSA with the Pierce Antibody Clean-up Kit (Product No. 44600)
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 decreased F(ab') ₂ activity	Use the Pierce IgG ₁ Fab and F(ab') ₂ Preparation Kit
Low F(ab') ₂ recovery	Incomplete washing of the pepsin resin	Two 500 μl washes of PBS are required for maximum recovery
A portion of undigested IgG does not bind to Protein A	Sample is goat or mouse IgG ₁	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 (Product No. 21001) before adding to the Protein A Column

Appendix

Recommended Digestion Times

This kit is for digesting 0.5 ml of IgG at 0.5-8 mg/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.

Table 1. Recommended digestion times for various species and concentrations of IgG.

Species	IgG (mg/ml)	Digestion Time
		(hours)
Rabbit	8	2
	3.5	1-2
	1.5	0.5
	0.5	0.5
Human	5.0	6-7
	2.5	3-4
	1.0	2-3
	0.5	1-2
Mouse	5.0	6-7
	2.5	2-3
	1.0	0.5-1
	0.5	0.5-1

Related Thermo Scientific Products

90009	Pierce Strong Cation Exchange Spin Column, Maxi, 8 spin columns and 16 collection tubes
90011	Pierce Strong Anion Exchange Spin Column, Maxi, 8 spin columns and 16 collection tubes
89868	Pierce Centrifuge Columns, 0.8 ml, 50 units
89956	NAb Protein A Spin Columns, 1 ml, 5 × 1 ml pre-packed columns for centrifuge or gravity-flow
44688	Pierce F(ab') ₂ Micro Preparation Kit
44985	Pierce Fab Preparation Kit, uses Immobilized Papain to prepare Fab fragments from IgG
44685	Pierce Fab Micro Preparation Kit
44980	Pierce IgG ₁ Fab and F(ab') ₂ Preparation Kit, uses Immobilized Ficin, optimized for mouse IgG ₁
44680	Pierce IgG ₁ Fab and F(ab') ₂ Micro Preparation Kit
23225	Pierce BCA Protein Assay Kit, sufficient to perform 500 standard tube assays
25200-25244	Precise™ Protein Gels (see catalog or website for a complete listing)
44600	Pierce Antibody Clean-up Kit

Cited References

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General References

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