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A Geno Technology, Inc. (USA) brand name

$F(ab)_2$ Fragmentation

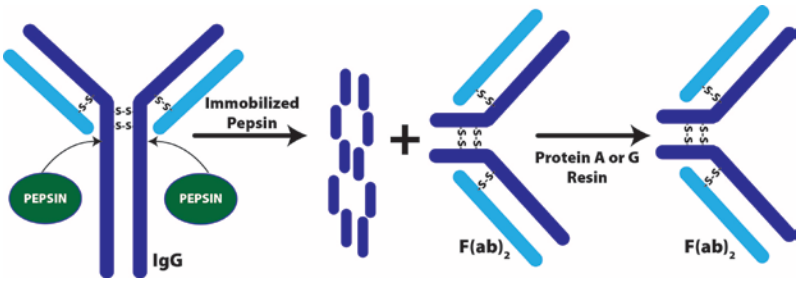
For the Generation of $F(ab)_2$ Fragments from IgG

(Cat. # 786-274, 786-864)

INTRODUCTION

The $F(ab)_2$ Fragmentation Kit is designed for the generation and isolation of $F(ab)_2$ fragments from IgG molecules. The kit utilizes our Immobilized Pepsin resin. Pepsin is a proteolytic enzyme that is routinely used for the generation of $F(ab)_2$ fragments from immunoglobulin G (IgG). The pepsin has the ability to cleave the heavy chains near the hinge region. One or more of the disulfide bonds that join the heavy chains in the hinge region are preserved, so the two Fab regions of the antibody remain joined together, yielding a divalent molecule (containing two antibody binding sites), hence the designation $F(ab)_2$. The light chains remain intact and attached to the heavy chain, whereas the Fc fragment is digested into small peptides. The Immobilized Pepsin offers the distinct advantage of eliminating enzyme contamination of the $F(ab)_2$ fragments. Following pepsin digestion the $F(ab)_2$ fragments are separated from undigested IgG and the large Fc fragments with the supplied Protein A or Protein G Spin Column. The Protein A or G Resin binds the IgG and Fc molecules and the $F(ab)_2$ are rapidly collected due to the spin-format design.

In addition, SpinOUT™ GT-600 desalting columns ensure the initial antibody sample is in the optimal condition for $F(ab)_2$ Fragmentation. The $F(ab)_2$ Fragmentation kit is optimized for mouse, rabbit and human IgG, using 0.25-4mg/ 0.5ml sample.



KIT COMPONENTS

Description	Cat. # 786-274	Cat. # 786-864
$F(ab)_2$ Digestion Buffer	60ml	60ml
Immobilized Pepsin	1.25ml resin	1.25ml resin
Phosphate Buffered Saline (PBS), [1X]	2 x 120ml	2 x 120ml
IgG Elution Buffer	100ml	100ml
Protein A Spin Column, 1ml	1 column	-
Protein G Spin Column, 1ml	-	1 column
Spin Column, 1ml	10	10
Caps	10	10
Rubber stoppers (Small)	10	10
SpinOUT™ GT-600, 3ml	10 columns	10 columns

STORAGE CONDITIONS

Kit is shipped at ambient temperature. Upon receipt store at 4°C, do NOT freeze.

SPECIFICATIONS

Immobilized Pepsin

- **Activity:**2-3mg Pepsin/ml resin
- **Support:** 6% Cross-linked Agarose

Protein A Resin

- **Capacity:** >20mg human IgG/ml resin
- **Support:** 6% highly cross-linked agarose
- See Appendix 1 for binding properties of Protein A and G.

Protein G Resin

- **Capacity:** 38mg human IgG/ml resin; >20mg sheep IgG/ml resin
- **Support:** 4% highly cross-linked agarose
- See Appendix 1 for binding properties of Protein A and G.

ADDITIONAL COMPONENTS

- 37°C waterbath
- 15ml collection tubes
- 2ml collection tubes

IMPORTANT

- The SpinOUT™ GT-600 columns will ensure the antibody is in the ideal buffering conditions, however if carrier proteins or protein stabilizers (i.e. BSA or gelatin) are present then we recommend using our Antibody Clean Up kit (Cat. # 786-803).
- The $F(ab)_2$ Fragmentation kit is optimized for mouse, rabbit and human IgG, using 0.25-4mg/ 0.5ml sample. Optimization may be required for other species antibodies. Also see Table 1, Appendix 1 for binding properties of Protein A and G.

PROTOCOL

Immobilized Pepsin Preparation

1. Suspend the resin by gently shaking and inverting the resin.
2. Transfer 0.25ml of the slurry to a 1ml Spin column with a wide bore pipette tip.
3. Cap the column, snap off the bottom tab and retain the end cap. Place into a 2ml collection tube.
4. Centrifuge the column at 3,000-5,000xg for 1 minute and discard the storage buffer.
5. Equilibrate the resin with the addition of 0.5ml Digestion Buffer.
6. Centrifuge the column at 3,000-5,000xg for 1 minute and discard the wash buffer. Repeat steps 5 and 6 once.
7. Cap the bottom of the column with the end cap from step 3.

IgG Sample Preparation

1. Place the SpinOUT™ column in a 15ml collection tube and centrifuge at 1,000g for 1 minute to compact the resin.
2. Prepare the SpinOUT™ column by removing the top and then bottom caps. Place into a 15ml collection tube.
3. Mark one side of the column and ensure in all centrifugations the mark is facing outwards during centrifugation.
4. Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer. This compacts the resin and removes the storage buffer.
5. Discard the storage buffer and return the SpinOUT™ GT-600 column in a 15ml collection tube.
6. Add 1ml Digestion Buffer into to the column.
7. Centrifuge the column at 1,000g for 2 minutes to remove the buffer.
8. Repeat steps 6 and 7 three more times, ensuring the buffer is discarded each time.
9. Place the column in a new collection tube.
10. Slowly, apply the 0.5ml IgG solution to the center of the SpinOUT™ resin.
11. Centrifuge the column at 1,000g for 2 minutes to collect the IgG solution. Discard the column.
12. Ensure the sample is at a final volume of 0.5ml, if not adjust with Digestion Buffer.

F(ab)$_2$ Fragment Generation

1. Add 0.5ml IgG sample to the equilibrated Immobilized Pepsin in the spin column.
2. Seal the spin column and incubate at 37°C in a high speed shaking waterbath or a 37°C incubator on an end-over-end mixer.
3. Incubate for the recommended digestion times:

Species	IgG (mg)	Digestion Time (hrs)
Rabbit	4	2
	2	1-2
	0.75	0.5
Human	2.5	6-7
	1.25	3-4
	0. 5	2-3
	0.25	1-2
Mouse	2.5	6-7
	1.25	2-3
	0.5	0.5-1

4. Remove the bottom cap and place the spin column into a 2ml Collection Tube. Centrifuge at 5,000xg for 1 minute to collect the digested antibody.
5. Place the spin column in a fresh collection tube and add 0.5ml PBS to the column. Centrifuge at 5,000g for 1 minutes. Repeat this step once.
6. Combine the washes and the digested antibody to give a final volume of 1.5ml.
7. Discard the used Immobilized Pepsin.

F(ab)$_2$ Purification

1. Allow the 1X PBS, Protein A or G Spin Column and IgG Elution Buffer to warm to room temperature before use.
2. Loosen the top cap and snap of the bottom closure of the Protein A or G Spin Column. Place in a 15ml Collection tube and centrifuge at 1,000xg for 1 minute to remove the storage buffer.
3. Equilibrate the column by adding 2ml 1X PBS to the column and centrifuging at 1,000xg for 1 minute. Repeat this step once.
4. Apply the rubber stopper to the bottom of the column and then apply the1.5ml sample to the resin. Tightly seal the column.
5. Incubate the column at room temperature with end-over-end mixing for 10-15 minutes.
6. Loosen the top cap and remove the bottom closure of the Protein A or G Spin Column. Place in a 15ml Collection tube and centrifuge at 1,000xg for 1 minute to collect the F(ab)$_2$ containing flow through.
7. For maximum F(ab)$_2$ fragment recovery, wash column with 1ml 1X PBS and combine with the initial F(ab)$_2$ fragment flow through from step 6. Repeat this step once.

8. Apply 1ml IgG Elution Buffer to the Protein A or G Spin Column and centrifuge at 1,000xg for 1 minute. Repeat his step a further two times. These fractions contain the Fc fragments and the undigested IgG. If the Fc fragments and the undigested IgG are to be retained, add 80µl 1M Tris pH8 to each of the fractions.
9. The protein concentration (mg/ml) can be determined by measuring absorbance at 280nm and dividing by an absorbance coefficient of 1.4. The NI™ Protein Assay (Cat. # 786-005) can also be used to determine protein concentration.

Protein A or G Spin Column Regeneration

1. Add 3ml IgG Elution Buffer and centrifuge for 1 minute at 1,000xg. Discard flow through. Repeat this step once.
2. Add 3ml 1X PBS and centrifuge for 1 minute at 1,000xg. Discard flow through. Repeat three times.
3. For storage at 3ml 1X PBS supplemented with a final concentration of 0.02% sodium azide as a preservative. Seal the column and store upright at 4°C.
4. The Protein A or G resin can be regenerated a maximum of 10 times.

TROUBLESHOOTING

Issue	Reason	Possible Solution
Low quantity of $F(ab)_2$ visualized by SDS-PAGE	Initial IgG sample contained interfering agents	Use our Antibody Clean Up kit or dialyze/ buffer exchange the IgG sample
	Essential cysteine has oxidized	Prepare Cysteine Digestion Buffer immediately prior to use and do not make stock solutions
	Reducing SDS-PAGE used	Use loading buffer without reducing agents
$F(ab)_2$ has low immunoreactivity	Digestion was too long	Reduce digestion time and ensure digestion times are always <20 hours
Undigested IgG and/or Fc domain present in $F(ab)_2$ fragment	The IgG species has low affinity for Protein A or G resin	See Appendix 1 for binding affinities. Use an alternative purification method, such as Protein A or G or ion-exchange

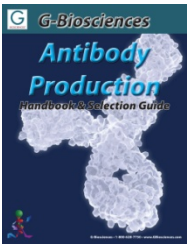
APPENDIX 1: BINDING PROPERTIES OF PROTEIN A AND PROTEIN G

Species	Antibody Class	Protein A	Protein G
Mouse	Total IgG	++++	++++
	IgG_1	+	+++
	IgG_{2a}	++++	++++
	IgG_{2b}	++++	++++
	IgG_3	+++	+++
Human	Total IgG	++++	++++
	IgG_1	++++	++++
	IgG_2	++++	++++
	IgG_3	+	++++
	IgG_4	++++	++++
Rat	Total IgG	+	++
	IgG_1	-	+
	IgG_{2a}	-	++++
	IgG_{2b}	-	++
	IgG_{2c}	++	+++
Hamster	Total IgG	++	++
Guinea Pig	Total IgG	++++	++
Rabbit	Total IgG	++++	+++
Horse	Total IgG	++	++++
Cow	Total IgG	++	++++
Pig	Total IgG	+++	++
Sheep	Total IgG	+	++
Goat	Total IgG	+	++
Chicken	Total IgG	-	-

Table 1: Relative affinity of Protein A and Protein G for Immunoglobulins

RELATED PRODUCTS

Download our Antibody Production Handbook.



http://info.gbiosciences.com/complete-Antibody-Production-handbook/

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