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A Geno Technology, Inc. (USA) brand name

Fab Fragmentation (Micro)

For the Generation of Fab Fragments from IgG

(Cat. # 786-273)

INTRODUCTION

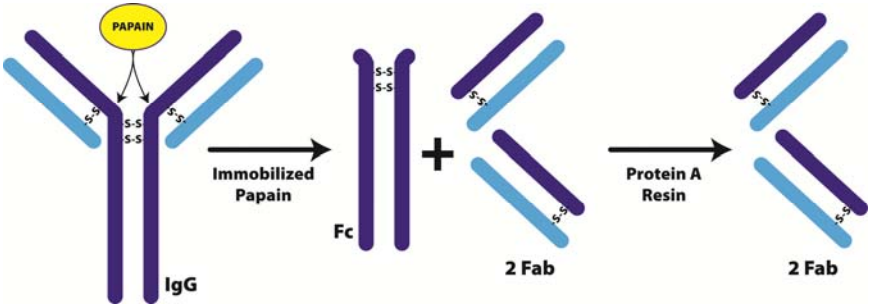
The Fab Fragmentation (Micro) Kit is designed for the generation and isolation of Fab fragments from IgG molecules.

The kit utilizes our Immobilized Papain resin. Papain is a cysteine protease enzyme (EC 3.4.22.2) that has the endopeptidase activity to cleave immunoglobulin G molecules in the hinge region. This cleavage results in the generation of three ~50kDa fragments; two Fab domains and a Fc domain. The papain-digested antibody is unable to promote agglutination, precipitation, opsonization, and lysis. Immobilized Papain offers the advantage of generating Fab and Fc fragments without the need to remove the papain enzyme after digestion.

Following papain digestion the Fab fragments are separated from undigested IgG and the Fc region with the supplied Protein A Spin Column. The Protein A Resin binds the IgG and Fc molecules and the Fab are rapidly collected due to the spin-format design.

In addition, SpinOUT™ GT-600 desalting columns are supplied to ensure the initial antibody sample is in the optimal condition for Fab Fragmentation.

The Fab Fragmentation (Micro) Kit is optimized for mouse, rabbit and human IgG, using 25-250µg/ 125µl sample. Suitable for 10 fragmentations.



ITEM(S) SUPPLIED (Cat. # 786-273)

Description	Size
Cysteine.HCl	0.5g
Fab Digestion Buffer	60ml
Immobilized Papain	0.5ml resin
Phosphate Buffered Saline (PBS), [1X]	120ml
IgG Elution Buffer	30ml
Protein A Spin Column (0.2ml resin)	1 column
Spin Column, 1ml	10
Caps	10
Rubber stoppers (Small)	25
SpinOUT™ GT-600, 1ml	10 columns

STORAGE CONDITIONS

Shipped at ambient temperature. Upon receipt store at 4°C, do NOT freeze.

SPECIFICATIONS

Immobilized Papain

- **Activity:** ≥15-40 BAEE units/ml resin (*One* unit *will hydrolyze 1.0 µmole of BAEE per minute at pH6.2 at 25°C*)
- **Capacity:** 250µg papain/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.

ADDITIONAL COMPONENTS

- 37°C Waterbath
- 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 Fab Fragmentation (Micro) Kit is optimized for mouse, rabbit and human IgG, using 25-250µg/ 125µl sample. Optimization may be required for other species antibodies. Also see Table 1, Appendix 1 for binding properties of Protein A.

PREPARATION BEFORE USE

1. ***Cysteine Digestion Buffer:*** Immediately prior to digestion, add Cysteine.HCl to the Fab Digestion Buffer to give a final concentration of 20mM and check the pH. The pH should be ~7.0, adjust if necessary. *Use 35mg Cysteine.HCl for every 10ml Fab Digestion Buffer.*

PROTOCOL

Immobilized Papain Preparation

1. Suspend the resin by gently shaking and inverting the resin.
2. Transfer 65µl 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 as 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 Cysteine 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 2ml 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. Return to the 2ml 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 to the 2ml collection tube.
6. Add 0.3ml Cysteine 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 after each centrifugation.
9. Place the column in a new collection tube.
10. Slowly, apply the 125µl 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 125µl, if not adjust with Cysteine Digestion Buffer.

Fab Fragment Generation

1. Add 125µl IgG sample to the equilibrated Immobilized Papain in the spin column.
2. Seal the spin column and incubate for 5-6 hours at 37°C in a high speed shaking waterbath or a 37°C incubator on an end-over-end mixer.
3. 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.
4. Place the spin column in a fresh collection tube and add 130µl PBS to the column. Centrifuge at 5,000g for 1 minutes. Combine the wash and the digested antibody to give a final volume of 255µl.
5. Discard the used Immobilized Papain.

Fab Purification

1. Allow the 1X PBS, Protein A 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 Spin Column. Place in a 2ml Collection tube and centrifuge at 1,000xg for 1 minute to remove the storage buffer.
3. Equilibrate the column by adding 0.4ml 1X PBS to the column and centrifuging at 1,000xg for 1 minute. Repeat this step three additional times.
4. Apply the rubber stopper to the bottom of the column and then apply the 255µl 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 Spin Column. Place in a 2ml Collection tube and centrifuge at 1,000xg for 1 minute to collect the Fab containing flow through.
7. For maximum Fab fragment recovery, wash column with 2 x 0.2ml 1X PBS and combine with the initial Fab fragment flow through from step 6.
8. Apply 0.4ml IgG Elution Buffer to the Protein A 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 40µl 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 can also be used to determine protein concentration.

Protein A Spin Column Regeneration

1. Add 0.4ml IgG Elution Buffer and centrifuge for 1 minute at 1,000xg. Discard flow through. Repeat two more times.
2. Add 0.4ml 1X PBS and centrifuge for 1 minute at 1,000xg. Discard flow through. Repeat two more times.
3. For storage at 0.4ml 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 resin can be regenerated a maximum of 10 times.

TROUBLESHOOTING

Issue	Reason	Possible Solution
Low quantity of Fab 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
Fab 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 Fab fragment	The IgG species has low affinity for Protein A resin, i.e. Goat and mouse IgG_1	See Appendix 1 for binding affinities. Use an alternative purification method, such as Protein G or ion-exchange

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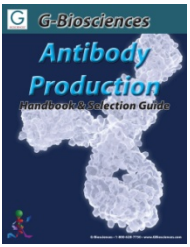
APPENDIX 1: BINDING PROPERTIES OF PROTEIN A AND PROTEIN G

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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