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A Geno Technology, Inc. (USA) brand name

Fab & F(ab)$_2$ Fragmentation of Mouse IgG$_1$ (Micro)

For the Generation of Fab or F(ab)$_2$ Fragments from Murine IgG$_1$

(Cat. # 786-277)

INTRODUCTION

The Fab & $F(ab)_2$ Fragmentation of Mouse IgG_1 kit is designed for the generation and isolation of Fab and $F(ab)_2$ fragments from mouse IgG_1 molecules.

The kit utilizes our Immobilized Ficin resin. Ficin (or Ficain) (~25,000Da) is a cysteine protease enzyme (EC 3.4.22.3) isolated from fig latex that has the endopeptidase activity to cleave immunoglobulin G molecules in the hinge region. Ficin has an effective range of pH4.0-9.5 with an optimal pH of 6.5 and cleaves bonds that involve uncharged or aromatic amino acids.

Ficin is typically used to cleave mouse IgG_1 as this are difficult to cleave with papain and pepsin. In the presence of 1mM or 10mM cysteine, ficin generates $F(ab')_2$ and Fab fragments respectively. Immobilized Ficin is a convenient reagent for producing Fab and $F(ab')_2$ fragments as it avoids the need to remove the ficin enzyme after digestion.

Following ficin digestion the fragments are separated from undigested IgG and the large Fc fragments with the supplied Protein A Spin Column. The Protein A Resin binds the IgG and Fc molecules and the Fab or $F(ab')_2$ 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 fragmentation.

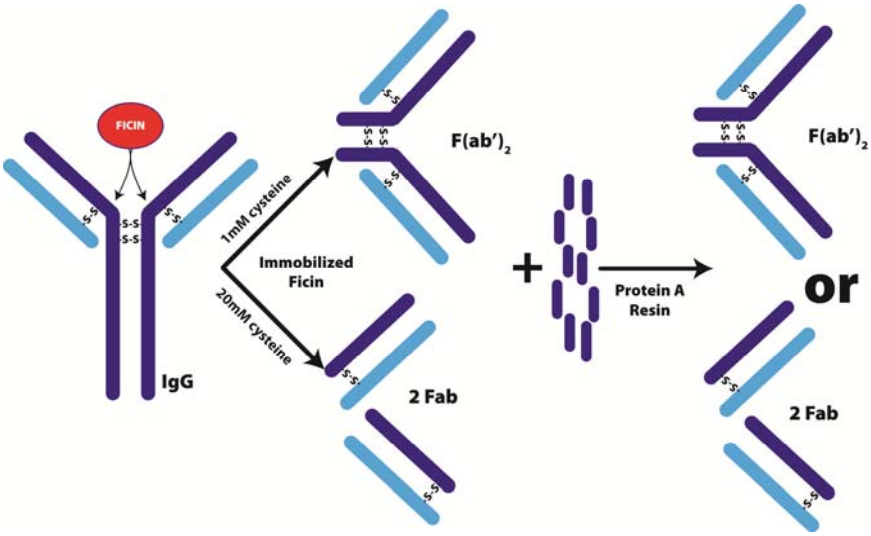
The Fab & $F(ab)_2$ Fragmentation of Mouse IgG_1 (Micro) kit is optimized for mouse IgG_1, using 25-250µg/ 125µl sample.

ITEM(S) SUPPLIED (Cat. # 786-277)

Description	Size
Cysteine.HCl	0.5g
IgG_1 Digestion Buffer	60ml
Immobilized Ficin	0.8ml resin
IgG Binding/ Wash Buffer	30ml
IgG Elution Buffer	30ml
Protein A Spin Column (0.2ml resin)	1 column
Rubber stoppers (Small)	10
Spin Column, 1ml	10
SpinOUT™ GT-600, 1ml	10 columns

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon receipt store at 4°C, DO NOT freeze.



SPECIFICATIONS

Immobilized Ficin

- **Activity:** 1-1.5mg ficin/ml of 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 ITEM(S) REQUIRED

- 37°C Waterbath/ incubator
- 2ml collection tubes

IMPORTANT INFORMATION

- 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 & $F(ab)_2$ Fragmentation of Mouse IgG_1 kit is optimized for mouse IgG_1, 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. ***Fab Digestion Buffer:*** Immediately prior to digestion, add Cysteine.HCl to the IgG_1 Digestion Buffer to give a final concentration of 20mM, check and adjust the pH to 5.5-6.0. *Use 35.2mg Cysteine.HCl for every 10ml IgG_1 Digestion Buffer.*
2. ***F(ab)$_2$ Digestion Buffer:*** Immediately prior to digestion, add Cysteine.HCl to the IgG_1 Digestion Buffer to give a final concentration of 1mM, check and adjust the pH to 5.5-6.0. *Use 1.76mg Cysteine.HCl for every 10ml IgG_1 Digestion Buffer.*

PROCEDURE

Immobilized Ficin Preparation

1. Suspend the resin by gently shaking and inverting the resin.
2. Transfer 0.2ml of the 33% slurry to a 1ml Spin column with a wide bore pipette tip to give 67µl settled resin.
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 appropriate Digestion Buffer (see Preparation Before Use).
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. Place into a2ml 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 compact the resin and removes the storage buffer.
5. Discard the storage buffer and return the SpinOUT™ GT-600 column in a 2ml collection tube.
6. Add 0.3ml appropriate 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 0.125ml 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 0.125ml, if not adjust with appropriate Digestion Buffer.

Fab or F(ab)$_2$ Fragment Generation

1. Add 0.125ml IgG sample to the equilibrated Immobilized Ficin 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 the column for 3-5 hours for Fab fragments and 24-30 hours for F(ab)$_2$ fragments. Ensure constant mixing is used throughout the incubations.
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 0.125ml IgG Binding/ Wash Buffer to the column. Centrifuge at 5,000g for 1 minutes. Repeat this step twice for a total of three washes. Combine the washes and the digested antibody to give a final volume of 0.5ml.
6. Discard the used Immobilized Ficin.

Fab or F(ab)$_2$ Purification

1. Allow the Protein A Spin Column, IgG Binding/ Wash Buffer 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 IgG Binding/ Wash Buffer 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 the 0.5ml 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 Spin Column. Place in a 2ml Collection tube and centrifuge at 1,000xg for 1 minute to collect the fragment containing flow through.
7. For maximum fragment recovery, wash column with 0.2ml IgG Binding/ Wash Buffer and combine with the initial Fab fragment flow through from step 6. Repeat this step once.
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 80µl 1M Tris pH8.0 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 Spin Column Regeneration

1. Add 0.4ml IgG Elution Buffer and centrifuge for 1 minute at 1,000xg. Discard flow through. Repeat this step once.
2. Add 0.4ml 1X PBS and centrifuge for 1 minute at 1,000xg. Discard flow through. Repeat three 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 or $F(ab)_2$ 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 Appropriate 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

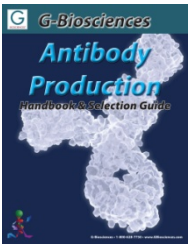
APPENDIX 1:BINDING PROPERTIES OF PROTEIN A AND PROTEIN G

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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