



FabALACTICA™

Fab Kit

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FOR RESEARCH USE ONLY

Instructions for Use

FabALACTICA™ Fab Kit Microspin 0.5 mg (A2-AFK-005)
Process 0.5 mg hlgG1

FabALACTICA™ Fab Kit Microspin 5×0.5 mg (A2-AFK-025)
Process 5 × 0.5 mg hlgG1

FabALACTICA™ Fab Kit Midispin 10 mg (A2-AFK-100)
Process 10 mg hlgG1

FabALACTICA™ Fab Kit Maxispin 100 mg (A2-AFK-1000)
Process 100 mg hlgG1



Preparations

Important Information

- Use lids and bottom caps during the incubation.
- Before centrifugation, remove the bottom cap (save the cap for Microspin) and loosen the lid (do not remove the lid).
- Bottom caps for Midi- and Maxispin columns are included.
- Seal caps and lids of Midi- and Maxispin columns with parafilm during the incubation to prevent leakage.

Additional Materials Required

- Reaction buffer: 150mM sodium phosphate, pH 7.0.¹
To ensure efficient digestion, it is important to use the recommended reaction buffer.
- PBS: 10mM sodium phosphate, 150mM NaCl, pH 7.4.
- Centrifuge tubes: 1.5-2 ml for Microspin, 15 ml for Midispin and 50 ml for Maxispin.

1. Optimal activity is obtained in 100-150mM sodium phosphate buffers at pH 6.5-7.5. Sodium chloride up to 150mM can be added without affecting the enzyme activity.

Above Hinge Digestion of Human IgG1 in Spin Columns

Protocol parameters for using the different product formats are given in Table 2.

Sample Preparation

Prepare the antibody in the reaction buffer according to Table 1.

Table 1. Preparation of hlgG1

	Microspin	Midispin	Maxispin
hlgG1 in buffer ¹	100 µl	1-2 ml ²	5-10 ml ²
Max amount hlgG1	0.5 mg	10 mg	100 mg

1. Equilibration

- 1.1 Break off the bottom cap of the FabALACTICA Immobilized column (save the cap for Microspin) and place the column in a centrifuge tube. Loosen the lid.
- 1.2 Centrifuge for 1 min to remove the storage solution. Discard the flow-through.
- 1.3 Equilibrate the column by adding reaction buffer and centrifuge for 1 min. Discard the flow-through.
- 1.4 Perform step 1.3 two additional times.
- 1.5 Insert the bottom cap.

2. Enzymatic Reaction

- 2.1 Add the antibody in a volume of reaction buffer according to Table 1.
- 2.2 Seal the column with the lid.
- 2.3 Fully suspend the media, mix by inversion and make sure there is a flow in the column.
- 2.4 Incubate the column with end-over-end mixing at room temperature overnight (16-18 h). A good mixing is important for optimal performance.

2. The digestion efficiency is reduced if the antibody concentration is below 5 mg/ml.

Table 2. Protocol Parameters for the Different Product Formats

	Microspin	Midispin	Maxispin
Storage Solution Removal			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	200 × g	100 × g	100 × g
Time	1 min	1 min	1 min
Equilibration			
Add buffer volume	300 µl (×3)	2.5 ml (×3)	10 ml (×3)
Spin	200 × g	100 × g	100 × g
Time	1 min	1 min	1 min
Enzymatic Reaction			
Incubation time ³	16-18 h	16-18 h	16-18 h
Collection of Processed Material			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	1000 × g	100 × g	100 × g
Time	1 min	2 min	2 min
For Maximum Recovery			
Add PBS volume	100 µl (×2)	1 ml (×2)	5 ml (×2)
Spin	1000 × g	100 × g	100 × g
Time	1 min	1 min	1 min

3. Collection of Processed Material

- 3.1 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.
- 3.2 Centrifuge according to Table 2 to collect the fragments.

4. For Maximum Recovery of the Sample

- 4.1 Insert the bottom cap.
- 4.2 Add PBS according to Table 2.
- 4.3 Seal the column with the lid and invert it a couple of times.
- 4.4 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.
- 4.5 Centrifuge according to Table 2 to collect the fragments.
- 4.6 Repeat steps 4.1-4.5.
- 4.7 Pool the collected fractions, including the sample from step 3.2.

3. The incubation time can be increased without overdigestion of the antibody.

Purification of Fab Fragments

Protocol parameters for using the different product formats are given in Table 3.

5. Equilibration

- 5.1 Break off the bottom cap of the CaptureSelect™ Fc column (save the cap for Microspin) and place the column in a centrifuge tube. Loosen the lid.
- 5.2 Centrifuge for 1 min to remove the storage solution. Discard the flow-through.
- 5.3 Equilibrate the column by adding PBS according to Table 3 and centrifuge for 1 min. Discard the flow-through.
- 5.4 Perform step 5.3 two additional times.
- 5.5 Insert the bottom cap. Apply parafilm around the bottom cap to prevent leakage.

Table 3. Protocol Parameters for use of CaptureSelect™ Fc Columns for the Different Product Formats

	Microspin	Midispin	Maxispin (per column)
Storage Solution Removal			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	200 × g	200 × g	200 × g
Time	1 min	1 min	1 min
Equilibration			
Add PBS volume	300 µl (×3)	3 ml (×3)	10 ml (×3)
Spin	200 × g	200 × g	200 × g
Time	1 min	1 min	1 min
Binding of Fc			
Incubation time	30 min	30 min	30 min
Collection of Fab			
Centrifuge tubes	1.5-2 ml	15 ml	50 ml
Spin	200 × g	200 × g	200 × g
Time	1 min	1 min	1 min
For Maximum Recovery of Fab			
Add PBS volume	100 µl (×2)	1 ml (×2)	2.5 ml (×2)
Spin 1	200 × g	200 × g	200 × g
Spin 2	1000 × g	200 × g	200 × g
Time	1 min	1 min	1 min

6. Binding of Fc Fragments

- 6.1 Add the pooled collected fractions from step 4.7 to the CaptureSelect™ Fc column⁴ and seal the column with the lid.
- 6.2 Fully suspend the media, mix by inversion and make sure there is a flow in the column.
- 6.3 Incubate the column with end-over-end mixing at room temperature for 30 min.

7. Collection of Fab Fragments

- 7.1 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.
- 7.2 Centrifuge according to Table 3 at $200 \times g$ for 1 min to collect the Fab fragments.

8. For Maximum Recovery of the Sample

- 8.1 Insert the bottom cap.
- 8.2 Add PBS according to Table 3.
- 8.3 Seal the column with the lid and invert it a couple of times.
- 8.4 Remove the bottom cap and place the column in a new centrifuge tube. Loosen the lid.
- 8.5 Centrifuge according to Table 3 to collect the Fab fragments.
- 8.6 Repeat steps 8.1-8.5.
- 8.7 Pool the collected Fab fragments, including the sample from step 7.2.

4. Note: For FabALACTICA Fab Kit Maxispin, two CaptureSelect™ columns are used in this section. Distribute the pooled collected fractions from step 4.7 evenly between the two columns.

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