

Human Fab Capture Kit

Instructions for Use

Product description

Product code: 28958325 (Human Fab Capture Kit)
29234601 (Human Fab Capture Kit, type 2)

Contents: The table below shows the contents of the kit.

Content	Human Fab Capture Kit	Human Fab Capture Kit, type 2
Human Fab Binder, 0.5 mg/mL in 0.15 M NaCl	50 μ L	80 μ L
Immobilization buffer, 10 mM Sodium acetate pH 5.0	2 \times 1.2 mL	2.6 mL
Regeneration solution, 10 mM Glycine-HCl pH 2.1	2 \times 90 mL	2 \times 120 mL

Storage: 2°C to 8°C

Kit capacity: The kit contains sufficient reagents for the following use:

- At least 10 immobilizations and 1000 regenerations with Human Fab Capture Kit
- At least 16 immobilizations and 1600 regenerations with Human Fab Capture Kit, type 2

Safety: For use and handling of the product in a safe way, refer to the Safety Data Sheet.

Note: *For research use only.*

Intended use

Human Fab Capture Kit and Human Fab Capture Kit, type 2 are intended for use in screening, such as simple response ranking or off-rate comparison, and kinetic characterization of human Fab antibody fragments in Biacore™ systems.

Human Fab Capture Kit, type 2 is designed for use with Biacore 8 series as the kit contains larger product volumes than Human Fab Capture Kit.

Human Fab Binder is immobilized on the sensor chip surfaces using the immobilization buffer included in the kit and Amine Coupling Kit. As a result, Fab fragments can be captured on the immobilized Human Fab Binder and studied for their interaction with the antigen. The surface is regenerated by removal of the captured Fab fragments and any associated molecules.

The kit is designed for use with Sensor Chip CM5.

Antibody specificity information

Human Fab Binder consists of two mouse monoclonal antibodies specific to kappa and lambda subtypes of human Fab fragments, respectively. The anti-kappa antibody is of subclass IgG1 and the anti-lambda antibody is of subclass IgG2a.

Fab subtype	Binding
Kappa 1	Yes
Kappa 2	Yes
Kappa 3	Yes
Kappa 4	Not tested
Lambda 1	Yes
Lambda 2	Not tested
Lambda 3	Yes

Cross-reactivity tests show that Human Fab binder binds specifically only to the Fab region of human antibodies. No binding to the Fc region has been observed. Human Fab Binder shows no detectable crossreactivity with Fab fragments from other species.

Required materials

See the list below for additional required materials (available from Cytiva).

- Sensor Chip CM5
- Amine Coupling Kit
- Running buffer (e.g., HBS-EP+)

Note: Refer to the *Instructions for Use for Sensor Chip CM5*.

Immobilization conditions

Antibody preparation

Centrifuge and mix Human Fab binder before use. Dilute the antibody to 20 µg/mL in immobilization buffer (e.g., 5 µL Human Fab binder + 120 µL immobilization buffer).

Active and reference surfaces

Immobilize the active and reference surfaces using the same settings for both flow cells.

Perform either:

- one immobilization in both flow cells (e.g., 1 and 2 in series).
- two separate immobilizations in two different flow cells, (e.g., 1 and 2 respectively).

Note: *Obtained immobilization levels in the active flow cell are expected to be slightly lower when the flow cells are immobilized in series. This is acceptable for most applications.*

For use on Biacore 4000, perform the immobilization in spots 1 + 2 and/or 5 + 4 in one injection by ticking the **Immobilize for capture** box in the immobilization wizard.

Note: *Do not use an unmodified surface as a reference.*

Immobilization settings

Reagents for immobilization are provided in the Amine Coupling Kit.

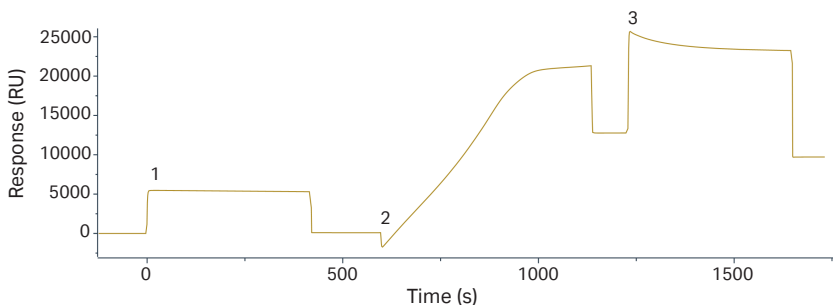
Perform immobilization at 25°C using a flow rate of 10 µL/min in systems where the flow rate can be adjusted. The immobilization procedure is shown in the table below.

Procedure step	Injection	Recommended conditions
Activation	EDC/NHS	<ul style="list-style-type: none"> All Biacore systems, except Biacore 4000: 7 minutes Biacore 4000: 10 minutes
Immobilization	Human Fab binder	<ul style="list-style-type: none"> Biacore T200, Biacore S200, Biacore 8 Series, Biacore 4000: 6 minutes Biacore 1 Series: 9 minutes Biacore X100: 8 minutes Biacore 3000: 5 minutes
Deactivation	Ethanolamine	7 minutes

Note: *These conditions are optimized to balance the immobilization of the two different antibody species of Human Fab Binder. Deviation from recommended immobilization conditions will shift the balance, which can impair the performance of the kit.*

This procedure typically results in immobilization levels above 5000 RU on Sensor Chip CM5.

The sensorgram below shows a typical immobilization sequence for Human Fab Binder on Sensor Chip CM5. The numbers indicate the start of injections of (1) EDC/NHS, (2) Human Fab Binder, and (3) Ethanolamine.



Recommended running conditions

Analysis temperature

Human Fab Capture Kit and Human Fab Capture Kit, type 2 are designed for use at 25°C.

Start-up cycles

For best assay performance, run at least one start-up cycle using identical settings as for the analysis cycles, including capture, analyte, and regeneration injections. Replace the analyte with running buffer.

Note: *Include both kappa and lambda Fab subtypes in the start-up cycle, either by running separate cycles or by using a mixture of both subtypes.*

Capture injection

For screening, inject the Fab sample using a contact time of 2 to 3 minutes at 10 $\mu\text{L}/\text{min}$. The contact time can be extended to increase the amount of Fab bound if required. These recommendations apply to samples containing about 0.5 to 10 $\mu\text{g}/\text{mL}$ Fab.

For kinetic characterization, follow the general recommendations for kinetic analyses in a Biacore system (refer to the Biacore system specific *Operating Instructions*). Adjust the Fab concentration and/or contact time to control the amount of Fab captured on the surface.

Analyte injection

For screening, inject the analyte using a contact time of 1 to 2 minutes at 10 $\mu\text{L}/\text{min}$. If you intend to rank Fabs by off-rate, include a blank cycle (with the analyte replaced by the running buffer) for each Fab sample to allow blank subtraction.

For kinetic characterization, follow the general recommendations for kinetic analyses in Biacore system (refer to the Biacore system specific *Operating Instructions*).

Regeneration injection

Regenerate the surface with two consecutive 1-minute injections of the regeneration solution provided in the kit at a flow rate of 10 to 30 $\mu\text{L}/\text{min}$. This will remove captured Fab fragments together with any analyte bound to them.

For more information on running conditions for different applications, guides, lab protocols, and free eLearnings, visit [cytiva.com/biacore](https://www.cytiva.com/biacore).



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28958220 AF V:9 02/2023