

Human Fab Capture Kit

Product description

Order code:	28-9583-25
Contents:	<ul style="list-style-type: none">• Human Fab Binder, 0.5 mg/ml in 0.15 M NaCl, 50 µl. Sterile filtered. No preservative added.• Immobilization buffer: 10 mM sodium acetate pH 5.0, 2 x 1.2 ml.• Regeneration solution: 10 mM glycine-HCl pH 2.1, 2 x 90 ml
Storage:	+2 to 8°C
Kit capacity:	The kit contains sufficient reagents for at least 10 immobilizations and 1000 regenerations.
Safety:	For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

Note: *For in vitro use only.*



Intended use

Human Fab Capture Kit is 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 Binder is immobilized on the sensor chip surfaces using the immobilization buffer included in the kit and Amine Coupling Kit. Fab fragments may then be captured on the immobilized Human Fab Binder and studied for their interaction with 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 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.

Framework	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.

Immobilization conditions

Required materials

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

- Carboxyl derivatized chip: Sensor Chip CM5, CM4, CM3 or C1
- Running buffer: (e.g. HBS-EP+, HBS-EP, HBS-P+, HBS-P, HBS-N, PBS-P+, PBS)
- Amine Coupling Kit

Preparation

Dilute Human Fab Binder to 20 µg/ml in immobilization buffer (5 µl Human Fab binder stock solution + 120 µl immobilization buffer).

Reference surface

The reference surface should be prepared in the same way as the active surface, i.e. the reference surface should be immobilized using the same settings as the active surface. For use on Biacore A100 and 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. For use on Biacore T200 and other instruments, perform two identical immobilizations in adjacent flow cells.

Note: *It is not recommended to use an unmodified surface as a reference.*

Immobilization settings

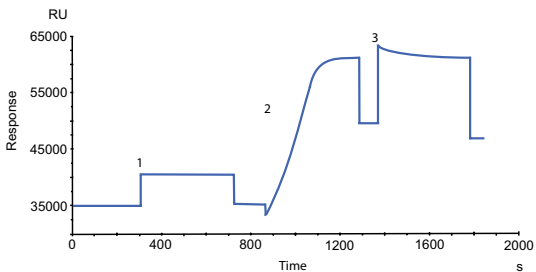
Perform immobilization at 25°C using a flow rate of 10 µl/min in systems where the flow rate can be adjusted.

Step	Injection	Conditions
Activation	EDC/NHS	<ul style="list-style-type: none">• Biacore A100 and Biacore 4000: 10 minutes• Other Biacore instruments: 7 minutes
Immobilization	Human Fab binder	<ul style="list-style-type: none">• Biacore 2000: 7 minutes• Biacore 3000: 5 minutes• Biacore 4000, Biacore A100, Biacore T200 and Biacore T100: 6 minutes• Biacore X100: 8 minutes
Deactivation	Ethanolamine	7 minutes

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

This procedure should result in immobilization levels of 9 000 to 13 000 RU.

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 is designed for use at 25°C.

Running buffer

HBS- EP+, available from GE Healthcare, is generally recommended as running buffer.

Start-up cycles

Run start-up cycles using the same settings as for analysis cycles, with human Fab captured on the surface and buffer injected instead of antigen. It is important to include both kappa and lambda Fab subtypes in the start-up cycle, either by running separate cycles or by using a mixture of subtypes.

Fab capture

For screening applications, inject the Fab sample using a contact time of 2 to 3 minutes at 10 $\mu\text{l}/\text{min}$. The contact time may 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 Biacore (see the documentation for your Biacore system). Adjust the Fab concentration and/or contact time to control the amount of Fab captured on the surface.

Antigen injection

For screening, inject antigen 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 comparison, include a blank cycle (with antigen replaced by buffer) for each Fab sample to allow blank subtraction.

For kinetic characterization, follow the general recommendations for kinetic analyses in Biacore (see the documentation for your Biacore system).

Regeneration

Regenerate the surface with two consecutive 1-minute injections of the the regeneration solution provided in the kit (10 mM glycine-HCl pH 2.1). This will remove captured Fab fragments together with any analyte bound to them.

For local office contact information, visit
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