

Human Antibody Capture Kit

Instructions for Use

Product description

Product code: BR100839 (Human Antibody Capture Kit)
29234600 (Human Antibody Capture Kit, type 2)

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

Content	Human Antibody Capture Kit	Human Antibody Capture Kit, type 2
Anti-Human IgG (Fc) antibody, 0.5 mg/mL in 0.15 M NaCl	50 µL	80 µL
Immobilization buffer, 10 mM Sodium acetate pH 5.0	1 mL	2.6 mL
Regeneration solution, 3 M MgCl ₂ pH 2.1	95 mL	2 × 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 Antibody Capture Kit
- At least 16 immobilizations and 1600 regenerations with Human Antibody 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 Antibody Capture Kit and Human Antibody Capture Kit, type 2 are intended for capture of human or humanized IgG antibodies as ligands in biomolecular interaction analyses using Biacore™ systems.

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

Anti-Human IgG (Fc) antibody is suitable for immobilization on sensor chip surfaces using the immobilization buffer included in the kit and Amine Coupling Kit. The regeneration solution is used for regeneration of the surface by removal of the captured antibodies.

Antibody information

Anti-Human IgG (Fc) is a monoclonal mouse anti-human IgG (Fc) antibody of IgG1 isotype. The antibody recognizes an epitope within the C_H2 domain in human IgG Fc of all subclasses, and it can also bind to monkey (Cynomolgus) and rabbit IgG.

Required materials

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

- Sensor chip (Sensor Chip CM5, CM4, CM3, or C1)
- Amine Coupling Kit
- Running buffer (HBS-EP+, HBS-P+, or HBS-N)

Note: Refer to the Instructions for Use for the respective sensor chip.

Note: Avoid using phosphate running buffers such as PBS in combination with Magnesium chloride (MgCl₂) as regeneration solution in order to avoid precipitation of Magnesium phosphate. For Biacore 8K and Biacore 8K+, the combination of MgCl₂ and phosphate running buffers results in a short life length of the syringe pump.

Recommended immobilization conditions

Antibody preparation

Centrifuge and mix Anti-Human IgG (Fc) before use. Dilute the antibody to 25 µg/mL in immobilization buffer (e.g., 5 µL Anti-Human IgG (Fc) + 95 µL immobilization buffer).

Active and reference surface

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

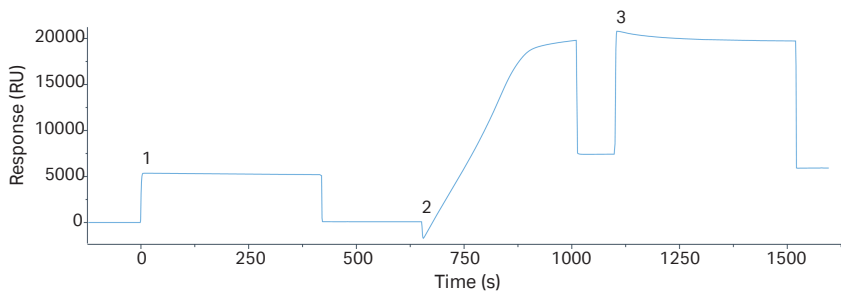
Reagents for immobilization are provided in the Amine Coupling Kit.

Perform immobilization at 25°C using a flow rate of 5 to 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	Anti-Human IgG (Fc)	<ul style="list-style-type: none">• All Biacore systems, except Biacore 3000 and Biacore 8 Series : 6 minutes• Biacore 3000: 3 minutes• Biacore 8K and Biacore 8K+: 8 minutes
Deactivation	Ethanolamine	7 minutes

This procedure typically results in immobilization levels above 5000 RU on Sensor Chip CM5. The exact amount of immobilized Anti-Human IgG (Fc) antibody is normally not critical for capturing antibodies. The immobilization level can be adjusted if necessary by adjusting the contact time or concentration of the Human IgG (Fc) antibody.

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



Recommended running conditions

Analysis temperature

Human Antibody Capture Kit and Human Antibody Capture Kit, type 2 are designed for use at 4°C to 40°C. Low analysis temperatures (<10°C) can require longer regeneration injections in order to completely remove any remaining antibodies from the surface.

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.

Capture injection

Inject antibody diluted to 1 to 10 µg/mL.

Ligand contact time and flow rate generally varies between 1 to 3 minutes and 5 to 10 µL/min, respectively.

Suitable capture levels depend on the application.

Analyte injection

Contact time and flow rate generally vary between 1 to 3 minutes and 10 to 30 µL/min, respectively.

Suitable analyte levels depend on the application.

Regeneration injection

Inject the regeneration solution using a contact time of 30 seconds at a flow rate of 10 to 30 µL/min. This will remove captured antibodies together with any analyte bound to them.

Select the **High viscosity solution** checkbox, and include a wash command (using running buffer) after the regeneration injection.

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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