GE Healthcare
Life Sciences

Instruction 22-0648-88 AD **Biacore™**

Human Antibody Capture Kit

Product description

Order code:	BR-1008-39
Contents:	• Anti-Human IgG (Fc) antibody: 0.5 mg/ml in 0.15 M NaCl, 50 µl Sterile filtered. No preservatives added. • Immobilization buffer: 10 mM sodium acetate pH 5.0, 1 ml • Regeneration solution: 3 M magnesium chloride, 95 ml
Storage:	+2 to 8°C
Kit capacity:	Human Antibody Capture Kit contains reagents sufficient 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 Antibody Capture Kit is intended for use in capture of human or humanized IgG antibodies as ligands in biomolecular interaction analyses using Biacore systems.

Anti-Human IgG (Fc) is suitable for immobilization on carboxyl derivatized surfaces using Amine Coupling Kit and the included Immobilization buffer. 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 is also shown to bind to monkey (Cynomolgus) and rabbit IgG.

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 Anti-Human IgG (Fc) to 25 µg/ml in Immobilization buffer (e.g. 5 µl Anti-Human IgG (Fc) + 95 µl 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

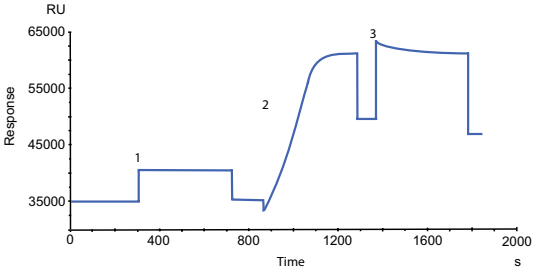
Perform immobilization at 25°C using a flow rate of 5 to 10 µl/min in systems where the flow rate can be adjusted.

Reagents for immobilization are provided in the Amine Coupling Kit.

Step	Injection	Conditions
Activation	EDC/NHS	• Biacore A100 and Biacore 4000: 10 minutes • Other Biacore instruments: 7 minutes
Immobilization	Anti-Human IgG (Fc)	• Biacore 3000: 3 minutes • Other Biacore instruments: 6 minutes
Deactivation	Ethanolamine	7 minutes

This procedure should result in immobilization levels of 9 000 to 14 000 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 may 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 is designed for use at 4°C to 40°C. Low analysis temperatures (<10°C) may require longer regeneration injections in order to completely remove any remaining antibodies from the surface.

Running buffer

HBS-EP+, HBS-EP, HBS-P+, HBS-P, HBS-N, PBS-P+, and PBS, available from GE Healthcare, are commonly used buffers for analysis.

Start-up cycles

For best assay performance, run at least one start-up cycle using identical settings as for the analysis cycles, including a human antibody sample and buffer instead of analyte.

Ligand injection

Contact time and flow rate generally varies between 1 to 3 minutes and 5 to 30 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 for example 20 µl/min. This will remove captured antibodies together with any analyte bound to them.

For local office contact information, visit
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