

Mouse Antibody Capture Kit

Instructions for Use

Product description

Product code: BR100838 (Mouse Antibody Capture Kit)
29215281 (Mouse Antibody Capture Kit, type 2)

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

Content	Mouse Antibody Capture Kit	Mouse Antibody Capture Kit, type 2
Anti-Mouse antibody, 1 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, 10 mM Glycine-HCl pH 1.7	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 Mouse Antibody Capture Kit
- At least 16 immobilizations and 1600 regenerations with Mouse 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

Mouse Antibody Capture Kit and Mouse Antibody Capture Kit, type 2 are intended for capturing mouse antibodies, which are used as ligands in various biomolecular interaction analyses in Biacore™ systems.

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

Anti-Mouse 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 removal of the captured antibodies.

Antibody information

Anti-Mouse antibody consists of polyclonal rabbit anti-mouse immunoglobulin antibodies mainly reacting with IgG. Reaction with other mouse antibody classes is expected but has not been tested.

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 (e.g., HBS-EP+, HBS-P+, HBS-N, PBS-P+, or PBS)

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

Recommended immobilization conditions

Antibody preparation

Centrifuge and mix Anti-Mouse antibody before use. Dilute the antibody to 30 µg/mL in immobilization buffer (e.g., 5 µL Anti-Mouse antibody + 162 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 in 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

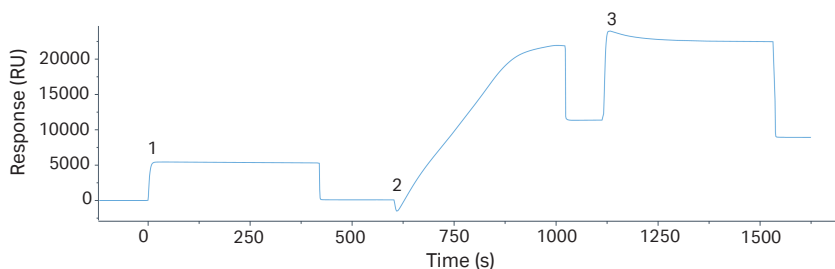
Reagents for immobilization are provided in the Amine Coupling Kit.

Perform the 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-Mouse anti-body	7 minutes
Deactivation	Ethanolamine	7 minutes

This procedure typically results in immobilization levels above 5000 RU on Sensor Chip CM5. The exact amounts of immobilized Anti-Mouse antibody are normally not critical for capturing antibodies. The immobilization level can be adjusted if necessary by changing the contact time or concentration of the Anti-Mouse antibody.

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



Recommended running conditions

Analysis temperature

Mouse Antibody Capture Kit and Mouse 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 3 minutes at flow rate of 10 to 30 µL/min. This will remove captured antibodies together with any analyte bound to them.

For antibodies where these conditions do not give adequate regeneration, an additional 30-seconds injection of 60 mM HCl or 10% formic acid can be tested.

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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