GE Healthcare
Life Sciences

His Capture Kit

Product description

Order code:	28995056
Contents:	• Anti-histidine antibody: 1 mg/ml in 0.15 M NaCl, 50 µl. Sterile filtered. No preservatives added. • Immobilization buffer: 10 mM sodium acetate pH 4.5, 1.2 ml. • Regeneration solution: 10 mM Glycine-HCl, pH 1.5, 100 ml.
Storage:	2°C to 8°C

For *in vitro* use only.

The kit contains reagents sufficient for 10 immobilizations and 1000 regenerations.

Intended use

His Capture Kit is intended for capture of histidine-tagged molecules as ligands in biomolecular interaction analyses using Biacore systems.

The Anti-histidine antibody 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 histidine-tagged ligand.

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1 Antibody information

The Anti-histidine antibody is a mouse monoclonal of IgG1 subclass that recognizes polyHistidine tags localized at the N- or C-terminus.

2 Recommended immobilization conditions

Required materials

Anti-histidine antibody and Immobilization buffer are included in the kit. Additional required materials (available from GE Healthcare) are:

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

Preparation

Dilute the Anti-histidine antibody to 50 µg/ml in Immobilization buffer (e.g. 5 µl Anti-histidine antibody + 95 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 the 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.

Procedure step	Injection	Recommended conditions
Activation	EDC/NHS	10 minutes in Biacore A100/4000, 7 minutes in other Biacore instruments
Immobilization	Anti-histidine antibody	7 minutes
Deactivation	Ethanolamine	7 minutes

This should typically result in immobilization levels of 9000 to 15000 RU on Sensor Chip CM5. The exact amount of immobilized Anti-histidine antibody is normally not critical for capturing ligand proteins. The immobilization level may be adjusted if necessary by adjusting the contact time or concentration of the Anti-histidine antibody

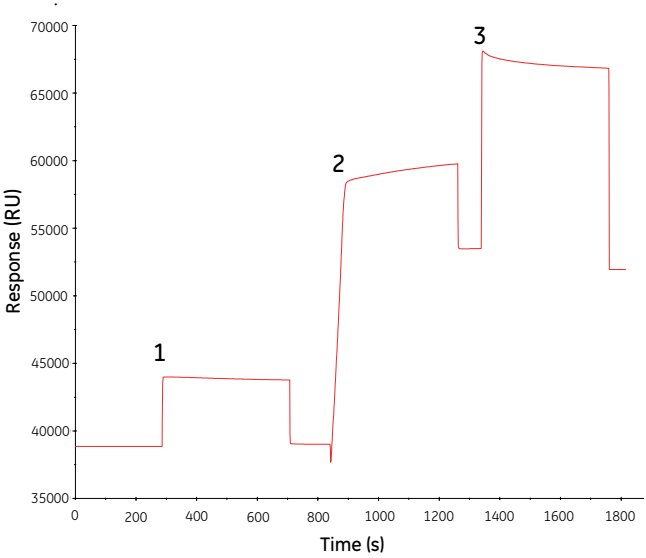

Fig 1. Typical sensorgram showing immobilization of Anti-histidine antibody on Sensor Chip CM5. The numbers indicate the start for the injections of 1) EDC/NHS, 2) Anti-histidine antibody and 3) ethanolamine.

3 Recommended running conditions

Analysis temperature

The His 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 ligands from the surface.

Running buffer

HBS-EP+, HBS-EP, HBS-P+, HBS-P, HBS-N, PBS-P+, 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 histidine-tagged ligand and buffer instead of analyte.

Ligand injection

Inject histidine-tagged ligands diluted to 1 to 10 µg/ml using a default contact time of 2 minutes at 10 µl/min. Suitable ligand levels depend on the application. Contact time and flow rate generally varies between 1 to 3 minutes and 5 to 30 µl/min respectively.

Analyte injection

Inject the analyte using a default contact time of 2 minutes at 30 µl/min. Suitable analyte levels depend on the application. Contact time and flow rate generally vary between 1 to 3 minutes and 10 to 30 µl/min respectively.

Regeneration injection

Inject the regeneration solution using a contact time of 1 minute at e.g. 30 µl/min. This will remove captured ligands together with any analyte bound to them.

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