

# His Capture Kit

## Instructions for Use

### Product description

Product code: 28995056 (His Capture Kit)  
29234602 (His Capture Kit, type 2)

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

Content	His Capture Kit	His Capture Kit, type 2
Anti-Histidine antibody, 1 mg/mL in 0.15 M NaCl	50 µL	90 µL
Immobilization buffer, 10 mM Sodium acetate pH 4.5	1.2 mL	2.6 mL
Regeneration solution, 10 mM Glycine-HCl pH 1.5	100 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 His Capture Kit
- At least 16 immobilizations and 1600 regenerations with His 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

His Capture Kit and His Capture Kit, type 2 are intended for capturing histidine-tagged molecules, which are used as ligands in biomolecular interaction analyses in Biacore™ systems.

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

The Anti-Histidine 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 histidine-tagged ligand and any associated molecules.

## Antibody specificity information

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

## 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-Histidine antibody before use. Dilute the antibody to 30 µg/mL in immobilization buffer (e.g., 5 µL Anti-Histidine 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

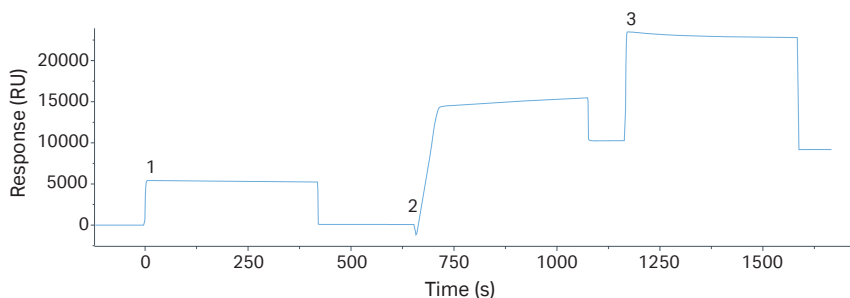
Reagents for immobilization are provided in the Amine Coupling Kit.

Perform 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"><li>All Biacore systems, except Biacore 4000: 7 minutes</li><li>Biacore 4000: 10 minutes</li></ul>
Immobilization	Anti-Histidine Antibody	7 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-Histidine Antibody is normally not critical for capturing ligand proteins. The immobilization level can be adjusted if necessary by adjusting the contact time or concentration of the Anti-histidine antibody.

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



# Recommended running conditions

## Analysis temperature

His Capture Kit and His 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 ligands 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 histidine-tagged ligands diluted to 1 to 10 µg/mL.

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

Suitable ligand levels depend on the application.

## Analyte injection

Contact time and flow rate generally vary between 1 to 3 min 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 1 minute and at a flow rate of 10 to 30 µL/min. This will remove captured ligands together with any analyte bound to them.

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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