Procedure

Capture of histidine-tagged molecules using Biacore His Capture Kit

This procedure provides recommendations for immobilization of histidine-tagged molecules (ligands) by capture to an anti-histidine antibody covalently coupled to a sensor chip surface (*see* Fig1). His Capture Kit is suitable for use with carboxyl-derivatized Biacore™ sensor chips and Series S sensor chips of the following series: Sensor Chip CM3, Sensor Chip CM4, Sensor Chip CM5, Sensor Chip C1, or Sensor Chip PEG.

The anti-histidine antibody (a mouse monoclonal of IgG1 subclass) provided in His Capture Kit is suitable for immobilization on carboxyl derivatized surfaces using Amine Coupling Kit and the included immobilization buffer.

Histidine capture generates a structural orientation of the histidine-tagged (his-tagged) ligand. Capture of the ligand occurs under near physiological conditions. With the included regeneration solution, the sensor chip can easily be regenerated after ligand capture to the anti-histidine antibody and reused.

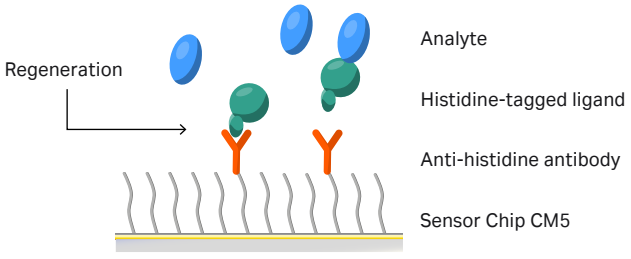

Fig 1. Schematic view of the chip surface of Sensor Chip CM5 with immobilized anti-histidine antibodies, histidine-tagged ligands captured to the antibodies and analyte binding to the hist-tagged ligands. The arrow indicates where the regeneration will act on the chip surface.

Immobilization settings

Perform the immobilization at 25°C using a flow rate of 5–10 µL/min. EDC, NHS, and ethanolamine are available in Amine Coupling Kit from Cytiva.

1. Dilute the anti-histidine antibody to 50 µg/mL in immobilization buffer (10 mM sodium acetate, pH 4.5).
2. Activate the chip surface by injection of EDC/NHS for 7 min (10 min in Biacore 4000).
3. Immobilize the anti-histidine antibody by injecting the anti-histidine antibody solution for 7 min.
   - The reference surface should be immobilized with anti-histidine antibody in the same way as the active surface. It is not recommended to use an unmodified surface as reference. In Biacore 4000, perform immobilization in spots 1 + 2 and/or 5 + 4. For use in Biacore T200 and other instruments, perform two identical immobilizations in adjacent flow cells.
4. Deactivate the chip surface by injection of ethanolamine for 7 min.

Note: *Using Sensor Chip CM5 typically results in immobilization levels of 9000 to 15 000 RU.*

Analysis cycle

Include at least one startup cycle before analyzing samples to allow the response to stabilize. Run the startup cycle over both active and reference surface. Use identical cycle settings as for the analysis cycles, including injection of his-tagged ligand, but with running buffer instead of analyte.

1. Capture the his-tagged ligand on the active surface by injecting the ligand solution (1–10 µg/mL in running buffer). Use contact time of 2 min and flow rate of 10 µL/min.
   - Suitable ligand levels depend on the application. Generally, contact times vary from 1–3 min and flow rates between 5 and 30 µL/mL.
2. Perform the interaction analysis by injecting the analyte for 2 min using flow rate 30 µL/mL.
   - Suitable analyte levels depend on the application. Generally, contact times vary between 1–3 min and flow rates between 10 and 30 µL/mL.
3. Regenerate the surface by injection of regeneration solution (10 mM Glycine-HCl, pH 1.5) for 60 s at, for example, 30 µL/mL to remove captured ligands together with any analyte bound to them.

Important considerations

- The exact amount of immobilized anti-histidine antibody is normally not critical for capturing ligands.
  - The immobilization level may be adjusted by changing the contact time or concentration of the anti-histidine antibody.
- His Capture Kit is designed for use at 4°C to 40°C. Low analysis temperatures (< 10°C) may require longer regeneration injection time in order to completely remove any remaining ligands from the surface.

Note: *All listed sensor chips are available from Cytiva*

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Ordering information

Product	Product code
His Capture Kit	28995056
His Capture Kit, type 2	29234602
Amine Coupling Kit	BR100050
Amine Coupling Kit, type 2, for Biacore 4000	BR100633

cytiva.com

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