

## Procedure

# Ligand thiol coupling to Biacore sensor chips

This guideline provides recommendations for immobilization of ligands containing free thiols by ligand thiol coupling (see Fig 1) to Biacore™ sensor chip surfaces. Ligand thiol coupling is suitable for carboxyl-derivatized sensor chips; Sensor Chip C1, Sensor Chip CM3, Sensor Chip CM4, Sensor Chip CM5, and Sensor Chip CM7.

## Required solutions

Required solutions are listed in Table 1. All reagents for ligand thiol coupling are available in the Thiol Coupling Kit. The PDEA Thiol Coupling Reagent is also available separately from Cytiva.

**Table 1.** Solutions required for immobilization of ligands by ligand thiol coupling

EDC	0.4 M of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in Milli-Q™ water
NHS	0.1 M of N-hydroxysuccinimide in Milli-Q water
PDEA <sup>1</sup>	80 mM of 2-(2-pyridinyldithio)ethaneamine in 0.1 M sodium borate, pH 8.5.
Ligand	Typically 20–50 µg/mL in immobilization buffer
Cysteine/NaCl	50 mM cysteine and 1 M NaCl in 0.1 M sodium acetate, pH 4.0

<sup>1</sup> Use within 30 min of preparation

## Suggested immobilization procedure

Follow the steps below to immobilize a ligand with ligand thiol coupling (see Fig 1). Perform the immobilization on the active surface. Use low flow rates, for example 5–10 µL/min in Biacore 3000 (in other instruments set by the immobilization wizard).

1. Activate the surface by injecting a mixture of EDC/NHS (1:1) for 2 min.
2. Introduce disulfide groups by injecting PDEA for 4 min.
3. Immobilize ligand by injecting the ligand solution for 6 to 7 min.
  - For detailed information on buffer and pH scouting refer to [cytiva.com](http://cytiva.com).
4. Deactivate excess reactive groups by injecting cysteine/NaCl for 4 min.

## cytiva.com

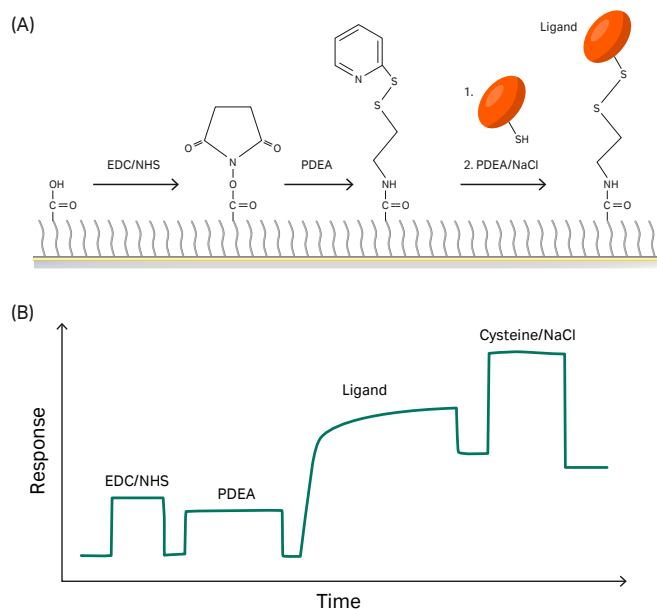
Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate. Biacore is a trademark of Global Life Sciences Solutions USA LLC or an affiliate doing business as Cytiva. Milli-Q is a trademark of Merck KGAA. All other third-party trademarks are the property of their respective owners.

© 2020 Cytiva

All goods and services are sold subject to the terms and conditions of sale of the supplying company operating within the Cytiva business. A copy of those terms and conditions is available on request. Contact your local Cytiva representative for the most current information.

For local office contact information, visit [cytiva.com/contact](http://cytiva.com/contact)

CY14998-21Oct20-PD



**Fig 1.** (A) The chemistry behind immobilization of ligands by ligand thiol coupling. (B) A typical sensorgram of a ligand immobilization using ligand thiol coupling.

## Important considerations

- Adjust immobilization levels by varying ligand concentration and contact time.
- Use a low flow rate to reduce ligand consumption.
- Recommended flow rates and contact times for optimal immobilization may vary between different Biacore systems.
- Running buffers can never be supplemented with a reducing agent (e.g., TCEP), since reducing agents will reduce PDEA prior to coupling. The coupling chemistry will then not work.

## Ordering information

Product	Product code
Thiol Coupling Kit	BR100557
PDEA Thiol Coupling Reagent	BR100058

