

## Procedure

# Amine coupling of ligand to Biacore sensor chips

This guideline provides recommendations for immobilization of ligands by amine coupling to Biacore™ sensor chips. Amine coupling is suitable for carboxyl-derivatized sensor chips and Series S sensor chips of the following series: Sensor Chip CM3, Sensor Chip CM4, Sensor Chip CM5, Sensor Chip CM7, Sensor Chip C1, and Sensor Chip PEG.

## Required solutions

Required solutions are listed in Table 1. Reagents for amine coupling are available in Amine Coupling Kit from Cytiva.

**Table 1.** Solutions required for immobilization of ligands by amine 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
Ligand	Typically 20–50 µg/mL in immobilization buffer
Ethanolamine	1 M ethanolamine-HCl, pH 8.5

## Suggested immobilization procedure

Follow the steps below to immobilize a ligand by amine coupling (see Fig 1). Perform the immobilization on the active surface. Use low flow rates from 5–10 µL/min.

1. Activate the surface by injecting a mixture of EDC/NHS (1:1) for 6–10 min (30 s for Sensor Chip PEG).
  - Using an EDC/NHS ratio of 20%/80% and 30 s activation time improves reproducibility for low ligand immobilizations (< 500 RU).
2. Immobilize the ligand by injecting the ligand solution for 5–10 min.
  - For detailed information on buffer and pH scouting refer to the Biacore Sensor Surface Handbook.
3. Deactivate excess reactive groups by injecting ethanolamine for 6–7 min.

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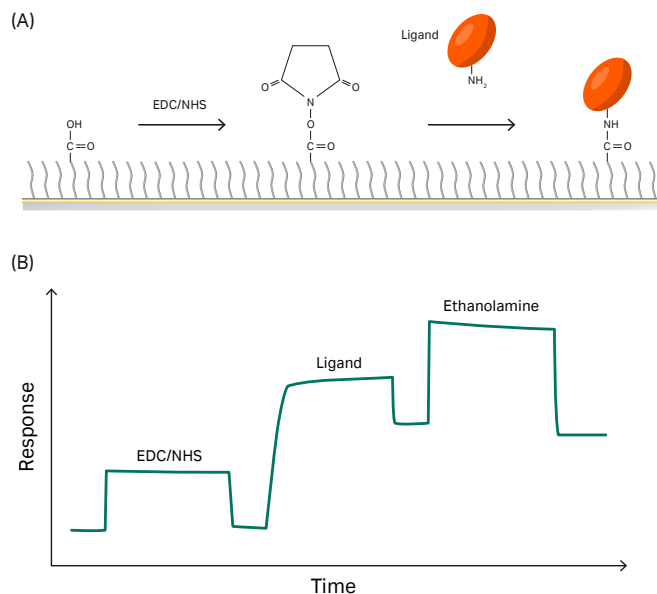
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**Fig 1.** (A) The chemistry behind immobilization of ligands by amine coupling. (B) A typical sensorgram of a ligand immobilization using amine coupling.

## 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.

## Ordering information

Product	Product code
Amine Coupling Kit, type 2 (Biacore 4000)	BR100633
Amine Coupling Kit (for all other Biacore systems)	BR100050

