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.

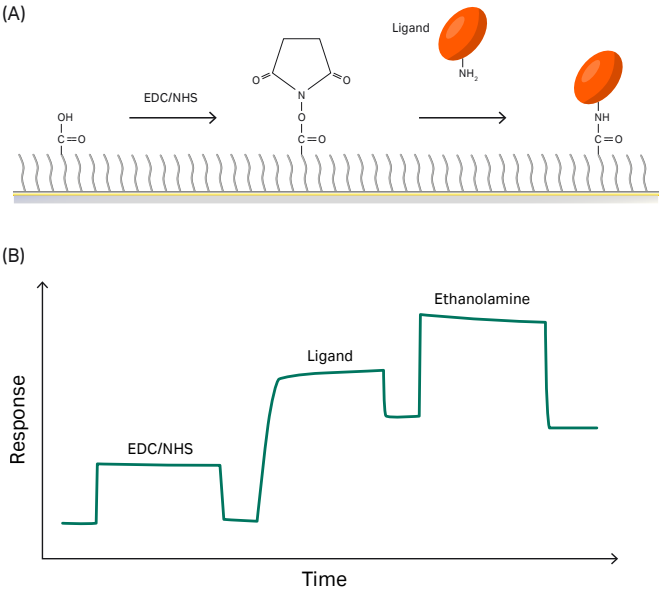


Fig 1. (A) The chemistry behind immobilization of ligands by amine coupling. (B) A typical sensorgram of a ligand immobilization using amine coupling.

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

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