

Amine coupling

This guideline provides recommendations for immobilization of ligands by amine coupling.

Required solutions

Required solutions are listed in table 1. Reagents for amine coupling are available in the Amine Coupling Kit from GE Healthcare.

Table 1. Solutions required for immobilization of ligands by amine coupling.

EDC	0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in Milli-Q water
NHS	0.1 M 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 Figure 1). Perform the immobilization on the active surface. Use low flow rates e.g. 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 6-10 minutes.
2. Immobilize the ligand by injecting the ligand solution for 5-10 minutes.
 - For detailed information on buffer and pH scouting refer to Sensor Surface Handbook.
3. Deactivate excess reactive groups by injecting ethanolamine for 6-7 minutes.

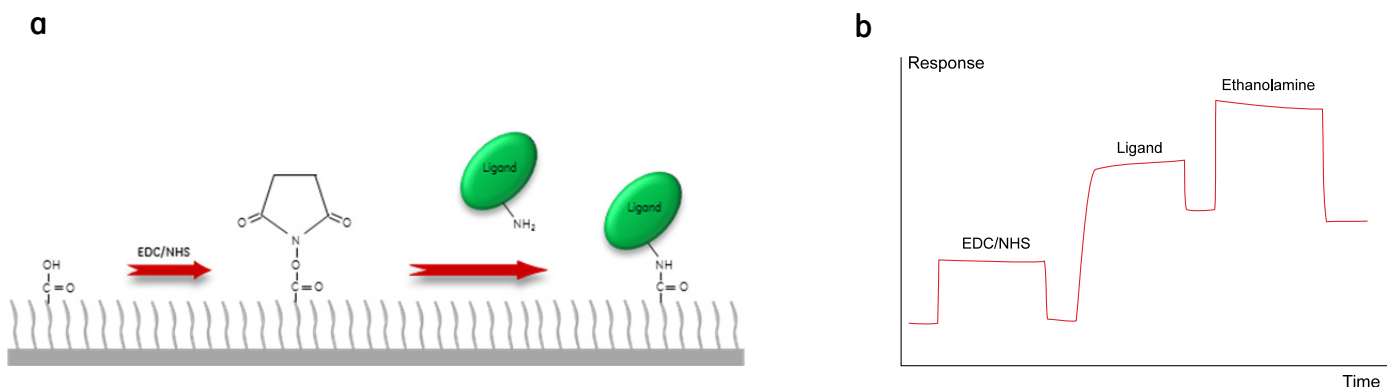


Figure 1. a) The chemistry behind immobilization of ligands by amine coupling. b) A typical sensorgram of a ligand immobilization using amine coupling.



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
- Amine coupling is suitable for carboxyl-derivatized sensor chip; Sensor CM3, Sensor Chip CM4, Sensor Chip CM5, Sensor Chip CM7 and Sensor Chip C1.

Ordering information:

Amine Coupling Kit, type 2
Amine Coupling Kit
Sensor Surface Handbook

Order code: BR-1006-33
Order code: BR-1000-50
Order code: BR-1005-71

Biacore 4000, Biacore A100 and Biacore S51
For all other Biacore systems

For local office contact information, visit
www.gelifesciences.com/contact

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