Procedure

Maleimide coupling to Biacore sensor chips using EMCH or BMPH

This procedure provides recommendations for immobilization of ligands containing free thiols on Biacore™ sensor chips by maleimide coupling using EMCH or BMPH. Maleimide coupling using EMCH or BMPH is suitable for carboxyl-derivatized sensor chips and Series S sensor chips of the following series: 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. EDC and NHS are available in Amine Coupling Kit from Cytiva. Cysteine and 1 M NaCl in 0.1 M sodium acetate, pH 4.0 are available in Thiol Coupling Kit.

Table 1. Solutions required for immobilization of ligands by maleimide coupling using EMCH or BMPH

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
Ethanolamine pH 7.0	1 M ethanolamine-HCl in 0.1 M sodium phosphate, pH 7.0
Borate 8.5 buffer	10 mM disodium tetraborate, pH 8.5 and 1 M NaCl
EMCH or BMPH	50 mM of N-[ε-maleimidocaprocic acid]-hydrazide or N-[β-maleimidopropionic acid]-hydrazide in Borate 8.5 buffer
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

Suggested immobilization procedure

Follow the steps below to immobilize a ligand by maleimide coupling using EMCH or BMPH (*see* Fig 1). Perform the immobilization on the active surface.

1. Activate the surface by injecting a mixture of EDC/NHS (1:1) for 7 min.
2. Introduce maleimide groups by injecting EMCH for 3 min.
3. Deactivate excess reactive groups by injecting ethanolamine pH 7.0 for 3 min.
4. Immobilize ligand by injecting the ligand solution for 6 to 7 min.
   - For detailed information on buffer and pH scouting refer to cytiva.com.
5. Deactivate excess reactive groups by injecting cysteine/NaCl for 4 min.

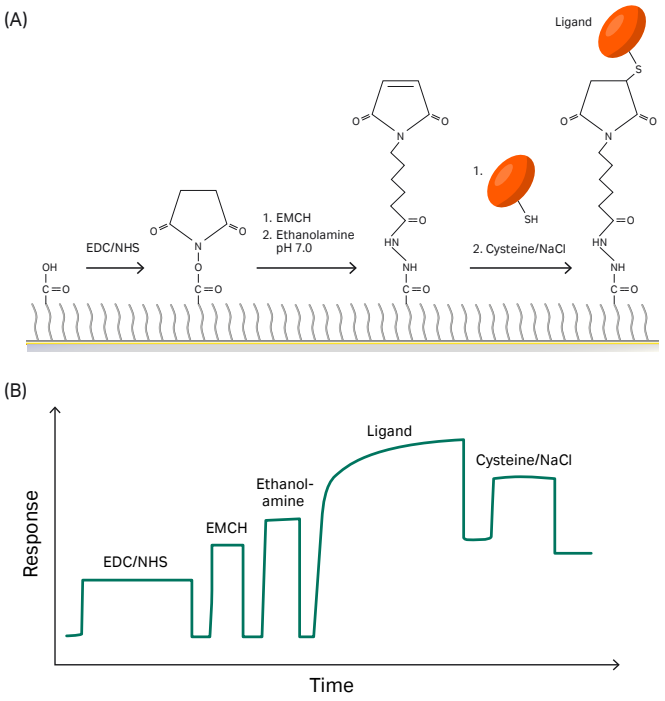

Fig 1. (A) The chemistry behind immobilization of ligands by maleimide coupling using EMCH or BMPH. (B) A typical sensorgram of a ligand immobilization by maleimide coupling using EMCH or BMPH.

Important considerations

- Neutral pH is important. Do not use the ethanolamine pH 8.5 that is included with Amine Coupling Kit since this will destroy the maleimide reagent on the dextran matrix.
- Centrifuge EMCH for 1 to 2 min at 10 000 to 20 000 × g before use. BMPH is more soluble in aqueous buffers and does not require centrifugation.
- 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 (for Biacore 4000)	BR100633
Amine Coupling Kit (for all other Biacore systems)	BR100050
Thiol Coupling Kit	BR100557

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