Procedure

Maleimide coupling to Biacore sensor chips using sulfo-GMBS

This guideline provides recommendations for immobilization of ligands containing free thiols to Biacore™ sensor chips by maleimide coupling using sulfo-GMBS. Maleimide coupling using sulfo-GMBS is suitable for carboxyl-derivatized sensor chip 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 the 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 from Cytiva.

Table 1. Solutions required for immobilization of ligands by maleimide coupling using sulfo-GMBS

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
Ethylenediamine	0.1 M ethylenediamine in 0.1 M sodium borate, pH 8.5
Sulfo-GMBS	50 mM of N-[γ-maleimidobutyryloxy]sulfo-succinimide ester 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

Suggested immobilization procedure

Follow the steps below to immobilize a ligand by maleimide coupling using sulfo-GMBS (*see* Fig 1). Perform the immobilization on the active surface. Use low flow rates as predefined in the Biacore method.

1. Activate the surface by injecting a mixture of EDC/NHS (1:1) for 6 to 7 min.
2. Introduce amine groups by injecting ethylenediamine for 6 to 7 min.
3. Introduce maleimide groups by injecting sulfo-GMBS for 4 min.
4. Immobilize ligand by injecting the ligand solution for 6 to 7 min.
   - For detailed information on buffer and pH scouting refer to the Biacore Sensor Surface Handbook.
5. Deactivate excess reactive groups by injecting cysteine/NaCl for 4 min.

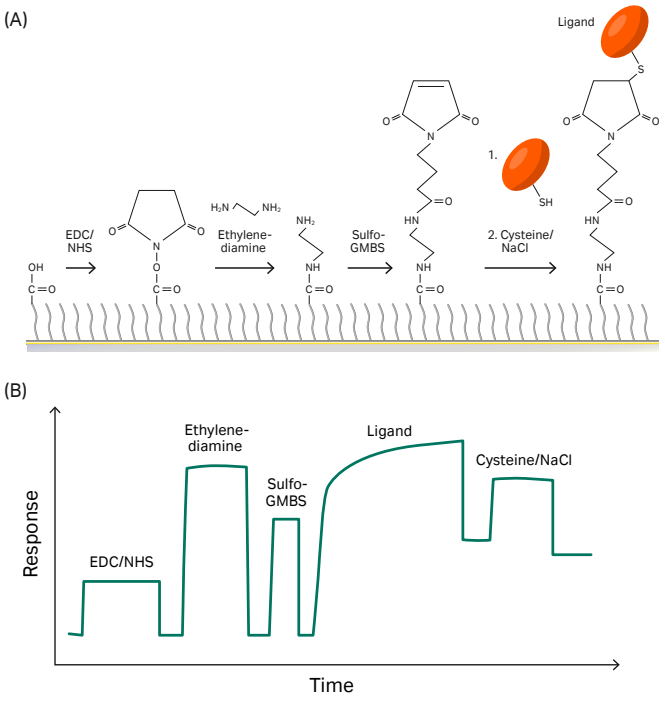

Fig 1. (A) The chemistry behind immobilization of ligands by maleimide coupling using sulfo-GMBS. (B) A typical sensorgram of a ligand immobilization by malemide coupling using sulfo-GMBS.

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 (for Biacore 4000)	BR100633
Amine Coupling Kit (for all other Biacore systems)	BR100050
Thiol Coupling Kit	BR100557

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CY15000-26Oct20-PD