

Maleimide coupling using EMCH or BMPH

This guideline provides recommendations for immobilization of ligands containing free thiols by maleimide coupling using EMCH or BMPH.

Required solutions

Required solutions are listed in table 1. EDC and NHS are available in the Amine Coupling Kit from GE Healthcare. Borate 8.5 buffer is also available from GE Healthcare.

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

EDC	0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in Milli-Q water
NHS	0.1 M 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 N-[ε-maleimidocaproic 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 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 7 minutes.
2. Introduce maleimide groups by injecting EMCH for 3 minutes.
3. Deactivate excess reactive groups by injecting ethanolamine pH 7.0 for 3 minutes.
4. Immobilize ligand by injecting the ligand solution for 6–7 minutes.
 - For detailed information on buffer and pH scouting refer to Sensor Surface Handbook.
5. Deactivate excess reactive groups by injecting cysteine/NaCl for 4 minutes.



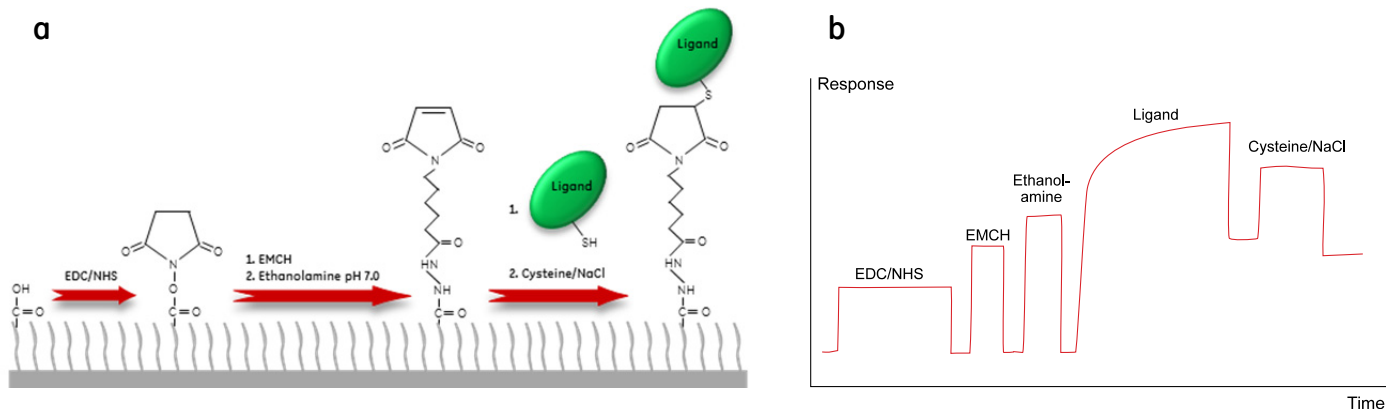


Figure 1. a) The chemistry behind immobilization of ligands by maleimide coupling using EMCH or BMPH. b) A typical sensorgram of a ligand immobilization by maleimide coupling using EMCH or BMPH.

Important considerations

- Neutral pH is important. Do not use the ethanolamine pH 8.5 that is included with the Amine Coupling Kit since this will destroy the maleimide reagent on the dextran matrix.
- Centrifuge EMCH for 1–2 minutes at 10 000–20 000xg 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
- Maleimide coupling using EMCH or BMPH is suitable for carboxyl-derivatized sensor chip; Sensor Chip CM3, Sensor Chip CM4, Sensor Chip CM5, Sensor Chip CM7 and Sensor Chip C1.

Ordering information:

Borate 8.5 buffer
 Amine Coupling Kit, type 2
 Amine Coupling Kit
 Sensor Surface Handbook

Order code: BR-1003-53
 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 systems

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

www.gelifesciences.com/biacore

GE Healthcare Bio-Sciences AB
 Björkgatan 30
 751 84 Uppsala
 Sweden

GE, imagination at work and GE monogram are trademarks of General Electric Company.

Biacore is a trademark of GE Healthcare companies.

All third party trademarks are the property of their respective owners.

© 2011 General Electric Company — All rights reserved.
 First published December 2003

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.

GE Healthcare UK Ltd
 Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA
 UK

GE Healthcare Bio-Sciences Corp
 800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327
 USA

GE Healthcare Europe GmbH
 Munzinger Strasse 5, D-79111 Freiburg,
 Germany

GE Healthcare Japan Corporation
 Sanken Bldg. 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073
 Japan

