GE Healthcare
Life Sciences

Instruction 22-0618-10 AB **Biacore™**

Thiol Coupling Kit

Product description

Order code:	BR-1005-57
Contents:	• Cystamine dihydrochloride, 90 mg • L-Cysteine, 61 mg • 1,4-Dithioerythritol (DTE), 154 mg • 1.0 M Ethanolamine-HCl, pH 8.5, 10.5 ml • 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 750 mg • N-Hydroxysuccinimide (NHS), 115 mg • 0.1 M 2-(4-Morpholino)ethanesulfonic acid (MES) pH 5.0, 100 ml • 2-(2-Pyridinyldithio)ethaneamine hydrochloride (PDEA), 100 mg • 0.1 M Sodium acetate 1.0 M, sodium chloride pH 4.0, 25 ml • 0.15 M Sodium borate pH 8.5, 25 ml
Storage:	+2 to 8°C
Capacity:	The kit contains sufficient reagents for approximately 50 surface thiol immobilizations and 10 PDEA ligand modifications, or approximately 15 ligand thiol immobilizations.

Safety:	For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

Note: *For in vitro use only.*

Kit information

Thiol Coupling Kit contains all reagents needed for the introduction of pyridyl disulfide groups into ligand molecules and for performing the surface and/or ligand thiol immobilizations on carboxyl derivatized surfaces (Sensor Chip CM5, CM7, CM4, CM3 and C1) in all Biacore systems.

Store the prepared reagent solutions at -20°C until used.

Note: *Running buffers must never be supplemented with a reducing agent (e.g. TCEP), since reducing agents will reduce PDEA and the coupling chemistry will not work.*

Reagent preparation

Surface thiol immobilization

Follow the instructions below to prepare 0.4 M EDC and 0.1 M NHS solutions.

Step	Action
1	Dissolve the EDC and NHS by adding 10.0 ml of deionized water to each vial.
2	Cap vials tightly and agitate until the solids are completely dissolved.
3	Dispense the EDC, NHS solutions separately in aliquots for storage at - 20°C. ***Note:*** *Use aliquots within two months.*

Follow the instructions below to prepare 40 mM cystamine dihydrochloride and 0.1 M DTE solutions.

Step	Action
1	Dissolve the cystamine dihydrochloride and DTE by adding 10.0 ml of the kit buffer 0.15 M sodium borate pH 8.5 to each vial.
2	Cap vials tightly and agitate until the solids are completely dissolved.
3	Dispense the cystamine dihydrochloride and DTE solutions separately in aliquots for storage at -20°C. ***Note:*** *Use aliquots within two months.*

Follow the instructions below to prepare a 20 mM PDEA-NaCl solution.

Step	Action
1	Dissolve 44 mg PDEA in 10.0 ml of the kit buffer 0.1 M sodium acetate, 1.0 M sodium chloride pH 4.0.
2	Cap vials tightly and agitate until the solids are completely dissolved.
3	Dispense the solution in aliquots for storage at -20°C. ***Note:*** *Use aliquots within two months.*

Ligand thiol immobilization

For preparation of EDC and NHS solutions, see *Surface thiol immobilization, on page 2*

Follow the instructions below to prepare a 50 mM L-cysteine solution.

Step	Action
1	Dissolve the L-cysteine by adding 10.0 ml of the kit buffer 0.1 M sodium acetate, 1.0 M sodium chloride pH 4.0.
2	Cap vials tightly and agitate until the solids are completely dissolved.
3	Dispense the L-cysteine solution in aliquots for storage at -20°C. ***Note:*** *Use aliquots within two months.*

Follow the instructions below to prepare a 120 mM PDEA solution.

Note: *This reagent solution must be mixed with borate buffer immediately before use.*

Step	Action
1	Dissolve 96 mg PDEA in 3.6 ml deionized water.
2	Cap vials tightly and agitate until the solids are completely dissolved.
3	Dispense the PDEA solution in aliquots for storage at -20°C. ***Note:*** *Use aliquots within two months.*

PDEA ligand modification

For preparation of EDC and NHS solutions, see *Surface thiol immobilization, on page 2*

Follow the instructions below to prepare a 15 mg/ml PDEA solution.

Step	Action
1	Dissolve 50 mg PDEA in 3.3 ml of the kit buffer 0.1 M MES pH 5.0.
2	Cap vials tightly and agitate until the solids are completely dissolved.
3	Dispense the solution in aliquots for storage at -20 °C. ***Note:*** *Use aliquots within two months.*

Immobilization procedures

Handling

- Thaw frozen aliquots and mix them gently to make sure that the solutions are homogeneous.
- EDC and NHS should be thawed and mixed immediately before use.
- PDEA diluted in borate buffer must be used within 30 minutes.
- The other thawed aliquots should be used during the day.
- Ethanolamine-HCl is used only when using Biacore 4000, Biacore A100, and Biacore S51.

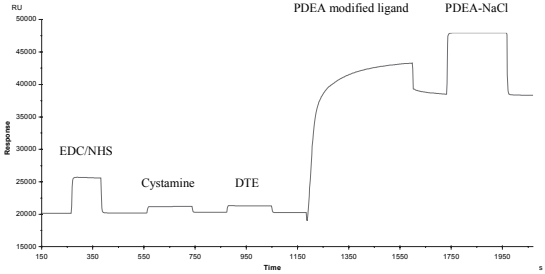
PDEA ligand modification

Step	Action
1	Prepare a ligand solution of 1 mg/ml, by dissolving 500 µg ligand in 0.5 ml 0.1 M MES pH 5.0.
2	Add 0.25 ml 15 mg/ml PDEA in 0.1 M MES.
3	Add 25 µl 0.4 M EDC.
4	Mix and allow to react for 10 min at 25°C or 1 hour on ice.
5	Remove the excess reagents by adding the sample volume to an illustra NAP-10 Column (GE Healthcare) or equivalent buffer exchange device equilibrated with suitable buffer.

Immobilization protocol for surface thiol coupling

Step	Action	Suggested contact time
1	Inject EDC and NHS 1:1 (v:v) mixture to activate the surface (mixture by means of system software).	2 minutes
2	Inject cystamine dihydrochloride to introduce disulfide groups.	3 minutes
3	Inject DTE to reduce disulfide groups.	3 minutes
4	Inject PDEA modified ligand.	7 minutes
5	Inject PDEA-NaCl to deactivate excessive reactive groups.	4 minutes

The sensorgram below shows a typical immobilization sequence for the surface thiol method.



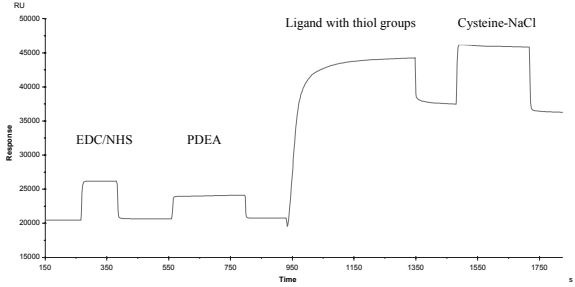
Refer to the instrument handbook for more general information.

Immobilization protocol for ligand thiol coupling

Step	Action	Suggested contact time
1	Inject EDC and NHS 1:1 (v:v) mixture to activate the surface (mixture by means of system software).	2 minutes
2	Mix PDEA (120 mM PDEA in water) and 0.15 M sodium borate buffer, 2:1 (v:v). This will give a final concentration of 80 mM PDEA in 50 mM sodium borate buffer pH 8.5. ***Note:*** *Mix just prior to use.*	-
3	Inject the PDEA solution to introduce disulfide groups	4 minutes
4	Inject ligand with thiol groups.	7 minutes
5	Inject cysteine-NaCl to deactivate excessive reactive groups.	4 minutes

Suitable flow rates are typically 5 to 10 µl/min.

The sensorgram below shows a typical immobilization sequence for the ligand thiol method.



Refer to the instrument handbook for more general information.

References

For more details on thiol coupling, refer to

- LabGuide - Ligand thiol coupling, and
- LabGuide - Surface thiol coupling,

available on www.biacore.com/applicationsupporttools - Methods.

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

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
United Kingdom

www.gelifesciences.com/sampleprep

First published Mar. 2003

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

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

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

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

imagination at work

22-0618-10 AB 06/2013