

Thiol Coupling Kit

Instructions for Use

Product description

Product code:	BR100557
Contents:	<ul style="list-style-type: none">• 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°C to 8°C
Capacity:	The kit contains reagents for approximately: <ul style="list-style-type: none">• 50 surface thiol immobilizations• 10 PDEA ligand modifications, OR• 15 ligand thiol immobilizations
Safety:	For safe use and handling of the product, refer to the Safety Data Sheet.

Note: *For research use only.*

Kit information

Overview

Thiol Coupling Kit is used for surface and ligand thiol immobilizations on Biacore™ sensor chips by introducing reactive disulfide groups or thiol groups, or both, into ligand molecules and the chip surface. The kit is used with acetate as specified below on carboxyl derivatized surfaces on all Biacore systems.

Required materials

The list below contains additionally required materials (available from Cytiva):

- carboxyl-derivatized chip (Sensor Chip CM7, CM5, CM4, CM3, or C1)
- 10 mM acetate, pH 4.0 to 5.5
- running buffer (for example, HBS-EP+, HBS-EP, HBS-P+, HBS-P, HBS-N, PBS-P+, or PBS)
- illustra™ NAP™-10 column for PDEA ligand modification

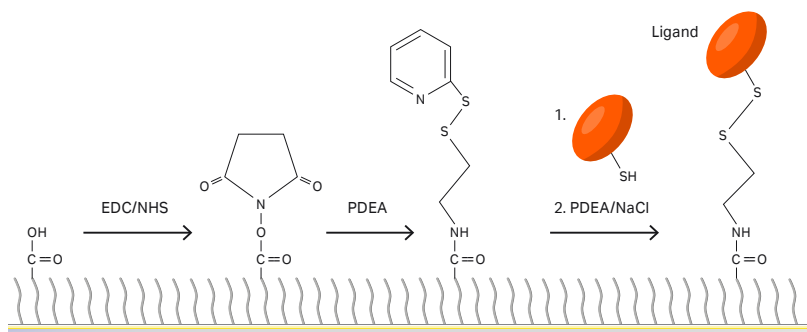
Note: Refer to the instructions for the respective sensor chip.

Chemistry

Ligand thiol coupling approach

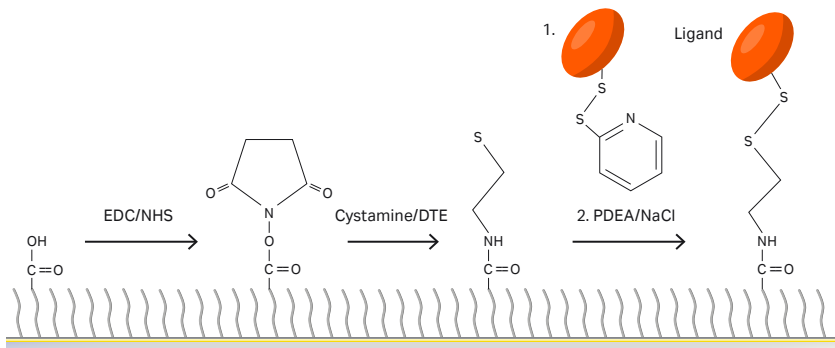
The ligand thiol coupling approach introduces reactive disulfide groups onto the sensor chip. Coupling occurs through thiol-disulfide exchange with native or introduced thiol groups on the ligand.

The image below illustrates the chemistry behind immobilization by ligand thiol coupling.



Surface thiol coupling approach

The surface thiol coupling approach introduces thiol groups onto the sensor chip and reactive disulfides into the ligand. Coupling occurs through thiol-disulfide exchange. The image below illustrates the chemistry behind immobilization by surface thiol coupling.



Ligand thiol immobilization preparations

Reagents list

The table below lists the reagents used for ligand thiol coupling.

Purpose	Reagent
Surface activation	0.4 M EDC and 0.1 M NHS
Introduction of reactive disulfide groups on chip surface	80 mM PDEA in 0.1 M sodium borate, pH 8.5
Deactivation of excess reactive groups on chip surface	50 mM L-cysteine and 1 M NaCl in 0.1 M sodium acetate, pH 4.0

Note: *The PDEA in sodium borate reagent solution is unstable and needs to be prepared immediately before the start of coupling, see [Prepare reagents for introduction of reactive disulfide groups, on page 4](#).*

Prepare reagents for surface activation

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

Step	Action
1	Dissolve the EDC by adding 10.0 mL of deionized water to the vial and do the same for the NHS.
2	Cap the vials tightly and vortex them until the solids are dissolved completely.
3	Dispense the EDC and the NHS solution each in individual aliquots for storage at -20°C.

Note:
Use aliquots within two months.

Prepare reagents for introduction of reactive disulfide groups

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

Note: *Immediately before immobilization, mix the reagent solution with borate buffer to a final concentration of 80 mM.*

Step	Action
1	Dissolve 96 mg PDEA in 3.6 mL deionized water.
2	Cap the vial tightly and vortex until the solids are dissolved completely.
3	Dispense the PDEA solution in aliquots for storage at -20°C.

Note:
Use aliquots within two months.

For the immobilization protocol, see [Immobilization protocol for ligand thiol coupling, on page 9](#).

Prepare reagents for deactivation of excess reactive groups

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 directly into the vial.
2	Cap the vial tightly and vortex until the solids are dissolved completely.
3	Dispense the L-cysteine solution in aliquots for storage at -20°C.

Note:

Use aliquots within two months.

Surface thiol immobilization preparations and ligand modification with PDEA

Reagents list

The table below lists the reagents used for surface thiol coupling.

Purpose	Reagent
Surface activation	0.4 M EDC and 0.1 M NHS
Introduction of disulfide groups on chip surface	40 mM cystamine dihydrochloride in 0.1 M sodium borate, pH 8.5
Reduction of disulfide groups to thiols on chip surface	0.1 M DTE in 0.1 M sodium borate, pH 8.5
Ligand modification with PDEA	15 mg/mL PDEA in 0.1 M MES, pH 5.0
Deactivation of excess reactive groups on chip surface	20 mM PDEA and 1 M sodium chloride in 0.1 M sodium acetate, pH 4.0

Prepare reagents for surface activation

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

Step	Action
1	Dissolve the EDC by adding 10.0 mL of deionized water to the vial and do the same for the NHS.
2	Cap the vials tightly and vortex them until the solids are dissolved completely.
3	Dispense the EDC and the NHS solution each in individual aliquots for storage at -20°C.
	Note: <i>Use aliquots within two months.</i>

Prepare reagents for introduction and reduction of disulfide groups

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

Step	Action
1	Dissolve the cystamine dihydrochloride by adding 10.0 mL of the 0.15 M sodium borate, pH 8.5, kit buffer to the vial.
2	Dissolve the DTE by adding 10.0 mL of the 0.15 M sodium borate, pH 8.5, kit buffer to the vial.
3	Cap the vials tightly and vortex them until the solids are dissolved completely.
4	Dispense the cystamine dihydrochloride solution in aliquots for storage at -20°C.
5	Dispense the DTE solution in aliquots for storage at -20°C.
	Note: <i>Use aliquots within two months.</i>

Prepare reagents for ligand PDEA modification

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 0.1 M MES, pH 5.0, kit buffer.
2	Cap the vial tightly and vortex until the solids are dissolved completely.
3	Dispense the solution in aliquots for storage at -20°C.

Note:
Use aliquots within two months.

Prepare reagents for deactivation of excess reactive groups

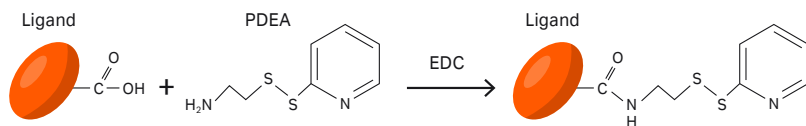
Follow the instructions below to prepare a 20 mM PDEA-NaCl solution.

Step	Action
1	Dissolve 44 mg PDEA in 10.0 mL of the 0.1 M sodium acetate, 1.0 M sodium chloride, pH 4.0, kit buffer.
2	Cap the vial tightly and vortex until the solids are dissolved completely.
3	Dispense the solutions in aliquots for storage at -20°C.

Note:
Use aliquots within two months.

Modify ligand with PDEA

Surface thiol immobilization requires ligand modification with PDEA. The image below illustrates the chemistry behind ligand modification with PDEA.



Follow the instructions below to modify the ligand with PDEA.

Step	Action
1	Prepare a ligand solution of 1 mg/mL, by dissolving 500 μg ligand in 500 μL 0.1 M MES, pH 5.0.
2	Add 250 μL 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 minutes at 25°C or for 1 hour on ice.
5	Remove excess reagents by adding the sample volume to an illustra NAP-10 Column (Cytiva) or equivalent buffer exchange device equilibrated with a suitable buffer.

Immobilization procedures

Handling

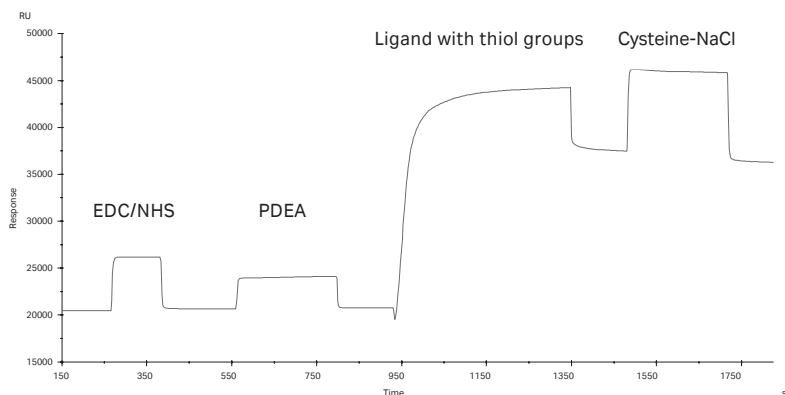
- Thaw frozen aliquots and mix them gently to make sure that the solutions are homogeneous.
- EDC and NHS must be thawed and mixed immediately before use.
- PDEA diluted in borate buffer must be used within 30 minutes.
- Other thawed aliquots must be used within a day.
- Running buffers must never be supplemented with a reducing agent such as TCEP, since reducing agents reduce PDEA so the coupling chemistry does not work.
- The ligand must be prepared in immobilization buffer, typically 20 to 50 $\mu\text{g}/\text{mL}$ in 10 mM sodium acetate, pH 4.0 to 5.5. For guidance on the selection of immobilization buffer, see *Selection guide, Biacore consumables* (CY14015).

Immobilization protocol for ligand thiol coupling

Step	Action
1	<p>Inject EDC and NHS 1:1 (v:v) mixture to activate the surface.</p> <p>Contact time: 2 minutes.</p> <p>Note: <i>When using wizards or predefined methods, the instrument mixes EDC and NHS. For manual runs, mix EDC and NHS by hand immediately before injection.</i></p> <p>Note: <i>A flow system wash with ethanolamine-HCl, pH 8.5, is included when using wizards or predefined methods, refer to the instrument software.</i></p>
2	<p>Immediately before use, mix PDEA (120 mM PDEA in water) and 0.15 M sodium borate buffer, 2:1 (v:v). This gives a final concentration of 80 mM PDEA in 50 mM sodium borate buffer, pH 8.5.</p>
3	<p>Inject the PDEA solution to introduce disulfide groups.</p> <p>Contact time: 4 minutes.</p>
4	<p>Inject ligand with thiol groups.</p> <p>Contact time: 7 minutes.</p>
5	<p>Inject cysteine-NaCl to deactivate excessive reactive groups.</p> <p>Contact time: 4 minutes.</p>

Suitable flow rates are typically 5 to 10 μ L/min.

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



Immobilization protocol for surface thiol coupling

Step	Action
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1 Inject EDC and NHS 1:1 (v:v) mixture to activate the surface.

Contact time: 2 minutes.

Note:

When using wizards or predefined methods, the instrument mixes EDC and NHS. For manual runs, mix EDC and NHS by hand immediately before injection.

Note:

A flow system wash with ethanolamine-HCl, pH 8.5, is included when using wizards or predefined methods, refer to the instrument software.

2 Inject cystamine dihydrochloride to introduce disulfide groups.

Contact time: 3 minutes.

3 Inject DTE to reduce disulfide groups.

Contact time: 3 minutes.

4 Inject PDEA modified ligand.

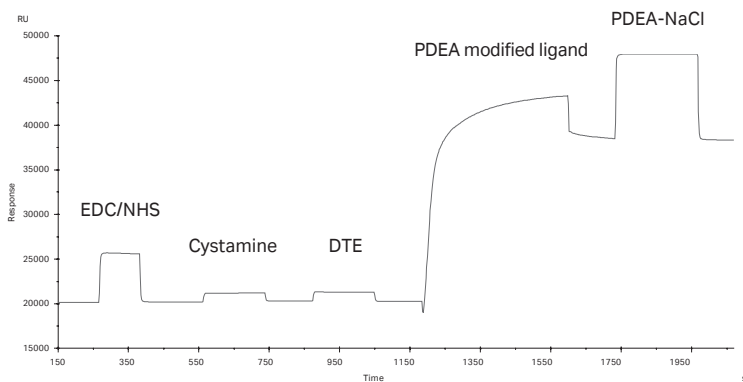
Contact time: 7 minutes.

Step	Action
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5	Inject PDEA-NaCl to deactivate excessive reactive groups.
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Contact time: 4 minutes.

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



References

For more details on thiol coupling, refer to the following lab procedures available on cytiva.com:

- *Ligand thiol coupling to Biacore sensor chips (CY14998)*
- *Surface thiol coupling of ligand to Biacore sensor chips (CY15004)*

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