Procedure

Biotinylation for Streptavidin or NeutrAvidin-biotin capture on Biacore™ sensor chips

This document describes how to biotinylate ligands for capture on Biacore Sensor Chip SA, Sensor Chip NA, and Sensor Chip CAP included in Biotin CAPture Kit. See *Ordering information* for product codes. The protocol is adapted for biotinylation of a 1.0 mg/mL (1 g/L) ligand solution using N-hydroxy succinimide ester biotin reagents (NHS-Biotin) and spin columns for buffer exchange. To fit other ligand concentrations or types of purification devices, the protocol needs to be rescaled.

Overview

Perform the following steps to biotinylate your ligand of interest:

- Prepare a 1 mg/mL solution of your ligand in an amine-free buffer.
- Calculate volume and concentration of biotinylation reagent.
- Prepare the NHS-Biotin stock solution.
- Mix ligand and NHS-Biotin and incubate.
- Purify the biotinylated ligand to remove excess non-reacted biotin.

Preparation of ligand

The ligand should be prepared in an amine-free buffer, for example 0.1 M sodium borate, pH 8.5. If the ligand buffer composition is unknown or contains amine components, such as for example Tris or sodium azide, perform a buffer exchange step prior to biotinylation. See *Ordering information* for recommended purification columns.

1. Prepare a 1.0 mg/mL (1.0 g/L) ligand solution. The volume of the ligand solution, V_{ligand} (L), should be based on type of purification column selected for the biotinylation.

2. Calculate how many moles of ligand ($Mole_{ligand}$) this corresponds to.

$$Mole_{ligand} = \frac{1.0\ (g/L) \times V_{ligand}\ (L)}{Mr_{ligand}\ (g/mole)}$$

Preparation of NHS-Biotin reagent

Select a suitable NHS-Biotin reagent for biotinylation. See *Ordering information* for recommended reagents. When performing biotinylation for capture on Biacore sensor chips, a lower excess of biotin should be used than what is generally recommended for NHS-Biotin reagents. A high excess of biotin will result in a high degree of modification which might have a negative impact on ligand function and activity. The recommendation is to use 1 M equivalent of biotin reagent or less. Some protein ligands are more difficult to biotinylate than others and some are more sensitive to covalent modification. This can sometimes motivate scouting the degree of biotinylation (molar ratio, e.g., R = 0.1, 0.3, 1, and 1.5, see below).

3. Calculate how many mole NHS-Biotin reagent is needed to obtain a biotin or ligand molar ratio of 1 or less in the final solution.

$$Mole_{biotin} = R \times Mole_{ligand}$$

where R = molar ratio of biotin to ligand. Choose a value ≤ 1.

4. Calculate volume of NHS-Biotin solution, V_{biotin} (L), to be added to the ligand solution. In this protocol the volume of NHS-Biotin to add to the ligand solution corresponds to 1/11 of the ligand solution volume to keep the volume of NHS-Biotin solution small in relation to the ligand solution.

$$V_{biotin}\ (L) = \frac{V_{ligand}\ (L)}{11}$$

5. Calculate required concentration of NHS-Biotin stock solution, $C_{biotin\ stock}$ (M).

$$C_{biotin\ stock}\ (M) = \frac{Mole_{biotin}}{V_{biotin}\ (L)}$$

6. Weigh a suitable amount of NHS-Biotin reagent. Note down weighed amount, $Amount_{biotin}$ (g).

Note: low mg amounts are sufficient.

7. Calculate volume of buffer or solvent, $V_{biotin\ stock}$ (L), required to obtain $C_{biotin\ stock}$ (M). This is dependent on the amount of NHS-Biotin weighed and the molecular weight of the biotinylation reagent, Mr_{biotin} (g/mole). See supplier recommendations for suitable solvents.

$$V_{biotin\ stock}\ (L) = \frac{Amount_{biotin}\ (g)}{Mr_{biotin}(g/mole) \times C_{biotin\ stock}\ (M)}$$

8. Prepare the NHS-Biotin stock solution by adding $V_{biotin\ stock}$ (L) of buffer or solvent to weighed NHS-Biotin, $Amount_{biotin}$ (g). Dissolve through gentle mixing.

Biotinylation

9. Add V_{biotin} (L) of NHS-Biotin stock solution to V_{ligand} (L) of 1 mg/mL ligand solution.

10. Incubate at 25°C for 1 h. If lower temperatures (on ice or at 4°C to 8°C) are used, incubate for 5 h. Incubation may also be performed overnight if convenient.

Purification of biotinylated ligand

11. Remove excess NHS-Biotin reagents from the ligand by using spin columns equilibrated according to vendor recommendation. Perform 2–3 consecutive desalting cycles using 2–3 different spin columns.

Example biotinylation

Table 1. Starting values example biotinylation

Parameters	Values
Volume 1 mg/mL ligand	50 µL
Molecular weight ligand	30 000 g/mole
Molar ratio biotin (R)	1
Molecular weight NHS-Biotin	341.4 g/mole
Weighted amount NHS-Biotin	1 mg

1. Prepare a 50 µL 1.0 mg/mL ligand solution.

2. Calculate how many moles of ligand this corresponds to.

$$Mole_{ligand} = \frac{1.0\ g/L \times 50 \times 10^{-6}\ L}{30\ 000\ (g/mole)} = 1.67 \times 10^{-9}\ mole$$

3. Calculate corresponding moles of biotinylation reagent (R = 1).

$$Mole_{biotin} = 1 \times 1.67 \times 10^{-9}\ mole = 1.67 \times 10^{-9}\ mole$$

4. Calculate volume of NHS-Biotin solution, V_{biotin} (L), to be added to the ligand solution.

$$V_{biotin}\ (L) = \frac{50 \times 10^{-6}\ L}{11} = 4.55 \times 10^{-6}\ L = 4.55\ \mu L$$

5. Calculate required concentration of NHS-Biotin stock solution, $C_{biotin\ stock}$ (M).

$$C_{biotin\ stock}\ (M) = \frac{1.67 \times 10^{-9}}{4.55 \times 10^{-6}} = 0.000367\ M$$

6. Weigh 1 mg of NHS-biotin.

7. Calculate volume of buffer/solvent, $V_{biotin\ stock}$ (L), required to obtain $C_{biotin\ stock}$ (M).

$$V_{biotin\ stock}\ (L) = \frac{0.002\ g}{341.4\ g/mole \times 0.000367\ M} = 0.008\ L = 8\ mL$$

8. Prepare NHS-Biotin stock solution by adding 8 mL buffer or solvent to 1 mg of NHS-Biotin. Mix gently until dissolved.

9. Add 4.55 µL NHS-Biotin stock to 50 µL 1.0 mg/mL ligand solution (L).

10. Incubate using selected conditions.

11. Purify to remove excess NHS-Biotin.

Tips

PBS buffer pH 7.4 can also be used for biotinylation following this protocol. Incubation overnight at 4°C to 8°C using PBS has shown to be comparable with incubation for 1 h at 25°C in 0.1 M sodium borate, pH 8.5.

The protocol can be rescaled if higher or lower ligand volumes are preferred.

Troubleshooting

Cause of low (capture or analyte binding) yield	Corrective action
Free biotin in ligand solution occupies binding sites on sensor chip which results in low capture levels	Repeat the biotin reagent removal step
Unsuitable biotinylation buffer: Buffer with amines will result in low capture level	Perform a buffer exchange step prior to the biotinylation
Unsuitable biotinylation buffer: Buffer with glycerol or too high salt concentration	Perform a buffer exchange step prior to the biotinylation. Alternatively prolong incubation time or scout for suitable biotin molar ratio.
Degree of biotinylation too low	Scout for different molar ratio of biotin to protein. If not sufficient, use different biotinylation chemistry and reagent.
Ligand concentration too low	Increase ligand concentration or optimize buffer exchange and purification (reagent removal) steps
Injection time too short	If a plateau is not reached during ligand injection, repeat ligand injection/inject more ligand over the same flow cell
Low ligand activity or low ligand availability/analyte binding	Decrease molar ratio of biotinylation reagent to ligand during biotinylation. Use NHS-Biotin reagents with a longer spacer. Consider using other biotinylation chemistry. Add additional purification step to remove remaining free biotin in the ligand solution.

Ordering information

Product	Vendor	Product code
Amersham™ MicroSpin™ G-50 columns, pack of 50	Cytiva	2753300
NHS-Biotin/Sulfo-NHS-Biotin	Thermo Scientific	20217/21217
NHS-LC-Biotin/Sulfo-NHS-LC-Biotin	Thermo Scientific	21336/21335
NHS-LC-LC-Biotin/ Sulfo-NHS-LC-LC-Biotin	Thermo Scientific	21343/21338
NHS-PEG4-Biotin	Thermo Scientific	21330
NHS-Biotin/Sulfo-NHS-Biotin	Merck/Sigma Aldrich	203112/B5161
NHS-dPEG 12-biotin	Merck/Sigma Aldrich	QBD10198
Series S Sensor Chip SA, pack of 3	Cytiva	BR100531
Series S Sensor Chip NA, pack of 3	Cytiva	29699622
Biotin CAPture kit, Series S	Cytiva	28920234

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