Procedure

Biotinylation for streptavidin/ neutravidin-biotin capture on Biacore sensor chips

This document covers recommendations for biotinylation of ligands to be captured on Biacore™ Sensor Chip SA, Sensor Chip CAP included in Biotin CAPture Kit and Series S Biotin CAPture Kit, respectively, and Series S Sensor Chip NA.

Protocol

Attachment of biotin to amine groups using NHS-based reagents. The protocol is valid for ligand concentrations of 0.1–1.0 mg/mL and adapted to ligand volumes suiting a desalting microspin column, with a total reaction volume of 100 µL (90 µL ligand + 10 µL biotin reagents).

1. Determine the ligand concentration (e.g., spectrophotometrically) if not known.
2. Dilute or dissolve the ligand (10–100 µg) in 90 µL of 0.1 M sodium borate, pH 8.5.
3. Weigh in the NHS-biotin reagents (X). Calculate the volume to dissolve the NHS-biotin reagents into (V_w). V_w results in the biotin concentration that corresponds to the biotin ratio 1.5 when 10 µL is added to the ligand. Follow the instructions from the supplier for choice of solvent.
4. Dissolve the NHS-biotin reagents, by adding the volume V_w.
5. Add NHS-biotin solution (immediately after preparation) to the ligand, to a molar excess or 1.5 (molar equivalent biotin/ligand).
6. 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 be performed overnight if convenient.
7. Remove excess reagents from the ligand by using desalting columns, equilibrated with a suitable buffer. If disposable columns are used, perform two to three consecutive desalting cycles.

$$V_w = (X \times M_{lg})/(M_w \times C_l \times 15)$$

V_w (mL) = Volume of solvent in which to dissolve the NHS-biotin reagent
X (mg) = Amount NHS-biotin reagent
M_{lg} = Molecular weight of the ligand
M_w = Molecular weight of NHS-biotin reagent
C_l (mg/mL) = Concentration of ligand in a 100 µL biotinylation volume

Important considerations

- If the ligand buffer composition is unknown or contains amine components (e.g., Tris, sodium azide), perform a buffer exchange step prior to biotinylation.
- A lower excess of biotin must be used than that generally recommended for NHS-biotin reagents. Use 1 molar excess of biotin reagents, 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 excess.

Note: *A lower excess of biotin must be used than that generally recommended for NHS-biotin reagents.*

- If disposable desalting column is used, always perform 2 (molar excess of biotin ≤ 2) or 3 (molar excess of biotin > 2 and ≤ 5) consecutive desalting cycles.
- If commercially biotinylated ligands are used, perform a desalting step prior to use.
- PBS buffer pH 7.4 can also be used for biotinylation following this protocol. Incubation overnight at 4°C to 8°C has shown to be comparable with incubation 1 h at 25°C with sodium borate, pH 8.5.

Troubleshooting tips

Cause of low (capture/ analyte binding) yield	Corrective action
Free biotin present in ligand solution	Repeat the biotin reagent removal step.
Unsuitable biotinylation buffer	Buffers with amines: buffer exchange step. Buffers with glycerol or high salt concentration: desalt or prolong incubation time or scout for molar ratio of biotin.
Degree of biotinylation too low	Increase molar ratio of biotinylation reagent to ligand or scout for molar ratio of biotin. If not sufficient, use different biotinylation chemistry and reagent.
Ligand concentration too low	Increase ligand concentration or optimize buffer exchange and reagent removal steps. If not possible, increase molar ratio of biotin or scout for molar ratio of biotin.
Injection time too short	If a plateau is not reached during ligand injection, inject more ligand over the same chip.
Low ligand activity or low accessibility of analyte bindning	Decrease the molar ratio of biotinylation reagent to ligand during biotinylation. Use NHS-biotin reagents with a longer spacer.

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Ordering information

Product	Product code
Sensor Chip SA, pack of 1	BR100398
Sensor Chip SA, pack of 3	BR100032
Series S Sensor Chip SA, pack of 3	BR100531
Series S Sensor Chip NA, pack of 1	29407997
Biotin CAPture Kit	28920233
Biotin CAPture Kit, Series S	28920234

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CY14992-21Oct20-PD