

NEUTRAVIDIN-MODIFIED SENSOR CHIPS

Instructions for use

PRODUCT DESCRIPTION

Product code

Prefix (designates the instrument): SCB, SCBS, SPP, SCBI, SPSM, SCH, SPMX, SCR, SCS, SD +
 Add: NAP, NAHLC30M, NAHLC200M, NAHCL1500M
 Example: SCBS NAHLC200M

Intended purpose

Capture immobilization of biotinylated biomolecules under physiological conditions with exceptionally high affinity. XanTec's NeutrAvidin™-modified sensor chips offer an efficient alternative to covalent immobilization, making it ideal for ligands that are challenging to immobilize or sensitive to covalent coupling.
 Recommended applications include the investigation of biomolecular kinetics involving proteins, peptides and nucleic acids.

Storage

Store at -20 °C, desiccated over molecular sieve 4A or at 2-8 °C in physiological buffer.

Related products

- Conditioning buffer 1, product code B C1-50ML

INTRODUCTION

The NAP and NAHLC sensor chips are coated with a bioinert, charge-reduced polycarboxylate matrix pre-functionalized with a 52 kDa NeutrAvidin™ tetramer. This immobilized NeutrAvidin™ effectively captures biotinylated biomolecules—such as biotinylated proteins, nucleic acids, and peptides—with exceptionally high affinity under physiological conditions.

NeutrAvidin™-modified sensor chips are a popular alternative to conventional amine coupling, particularly when standard amine chemistry poses challenges for ligand immobilization. These challenges often arise when the ligand is too acidic to efficiently pre-concentrate on a polycarboxylate surface or is unstable during the preconcentration process. NeutrAvidin™-functionalized surfaces offer several key advantages, including enhanced control over immobilization levels and the ability to capture ligands in an oriented manner using site-directed biotinylation strategies, such as AviTag™.

Compared to streptavidin-modified chips, NAHLC sensor chips offer the advantage of a lower overall negative charge density, attributed to the nearly neutral isoelectric point of NeutrAvidin™. When paired with the charge-reduced HLC base coating, these chips can show a significantly reduced level of nonspecific binding in the presence of positively charged biomolecules. Furthermore, the NeutrAvidin™-biotin complex is exceptionally stable and withstands most regeneration protocols. These properties make NeutrAvidin™-modified HLC chips highly versatile and ideal for a wide variety of experimental conditions and applications.

XanTec offers a range of NeutrAvidin™-modified sensor chips specifically designed to meet the needs of surface plasmon resonance (SPR) applications. The 2D NAP sensor chips provide superior diffusion characteristics, though they feature lower immobilization densities, making them an ideal choice for demanding biomolecular interaction analyses, particularly with bulkier analytes and weak binders that exhibit fast on- and off-rates. Conversely, NAHLC200M and NAHLC1500M sensor chips support higher immobilization levels, making them particularly well-suited for applications involving smaller analytes.

ADDITIONAL MATERIALS REQUIRED FOR BIOTIN CAPTURE IMMOBILIZATION

Biotinylated biomolecule (to be provided by the user)

Conditioning buffer 1 (product code B C1-50ML): 50 mM NaOH, 1 M NaCl

Optional: biotin blocking solution (to be provided by the user): 50 μM of a water-soluble biotin-derivate like biocytin or biotin-PEO₃-amine in running buffer. Free biotin is only poorly soluble in aqueous solutions and therefore, has a lower quenching efficiency.

PREPARATIONS FOR BIOTIN CAPTURE IMMOBILIZATION

Clean the SPR fluidics

Ensure that the flow system of your SPR equipment is free from any protein contamination, as even small amounts of desorbed protein can accumulate on the charged sensor surface. If necessary, clean the system using either 1 % Tween 20 or, for a more stringent cleaning, 0.5 % SDS for 5 minutes, followed by 50 mM glycine·HCl (pH 9.5) for 10 minutes (both included in the Desorb Kit, product code K D-500ML). The glycine is required to remove residual traces of SDS.

Ligand biotinylation

To ensure effective capture on XanTec's NeutrAvidin™-modified sensor chips, the ligand should be biotinylated at a substitution level of one biotin molecule per ligand or lower. Since commercial biotinylation protocols often result in higher degrees of labeling, it is recommended to use an NHS-biotin reagent at a concentration of 1.1 moles of biotin per mole of ligand or less.

It's crucial to thoroughly remove any unreacted biotinylation reagent from the ligand solution before capture to avoid competition with the biotinylated ligand for binding sites on the NeutrAvidin™ chip. This can be achieved through size-exclusion chromatography, and for optimal results, the purification process should be repeated at least once to ensure complete removal of free biotin.

Failure to properly remove excess biotin can significantly reduce binding efficiency, so taking these extra steps will help ensure robust and reliable immobilization.

Sensor chip

Allow the sealed sensor chip pouch to equilibrate at room temperature to prevent condensation on the chip surface.

After opening to pouch, install the sensor chip by following the instrument manufacturer's instructions.

Note: XanTec SPR sensor chips, like all nanocoatings, are prone to degradation when exposed to the atmosphere due to reactive oxygen species in the air. To prevent this, unmounted sensor chips should be stored in a closed container under an inert gas atmosphere or in a physiological buffer for short-term storage.

PROTOCOL FOR BIOTIN CAPTURE IMMOBILIZATION

| Procedure | Flowrate [μ L/min] | Injection time [s] |
|--|----------------------------|-----------------------|
| <p>1 Equilibrate your SPR-system with physiological running buffer like HBSTE or HBSTE+ and mount a compatible XanTec NeutrAvidin™ sensor chip.</p> | | |
| <p>2 Condition all channels with Conditioning buffer 1. Wait until the baseline has stabilized. Note: Shorter or no conditioning can increase the immobilization yield but results in a less stable baseline.</p> | 15 | 600 |
| <p>3 Divert your reference channel and inject your biotinylated ligand at concentrations of 1–100 nM. Low ligand concentrations and flow rates are recommended to achieve good control over ligand immobilization levels. Adjust conditions and repeat ligand injection as required to achieve the target immobilization level. Wait until the baseline has stabilized.</p> | 1–10 | 60–900 |
| <p>4 Optional: Inject biotin blocking solution to quench remaining NeutrAvidin™ binding sites. Note: In SPR instruments with serial flow cells, the biotin blocking solution may carry over to adjacent flow cells, potentially decreasing ligand immobilization capacity in these cells.</p> | 15 | 300 |
| <p>5 Start Interaction analysis. We recommend to begin with 3–5 consecutive regeneration cycles to improve data quality.</p> | | |

Notes

Tween containing running buffers like HBSTE or HBSTE+ are generally recommended for NeutrAvidin™-modified sensor chips from XanTec.

Avoid prolonged incubation of the sensor chip in water, as this can negatively affect the integrity of the sensor coating over time. Instead, use a physiological buffer like HBSTE.

REGENERATION

The selection of a suitable regeneration buffer is crucial when performing binding studies in which the analyte does not dissociate completely within an adequate period of time. In such cases, the analyte must be removed manually through a regeneration procedure. The goal is to ensure complete analyte removal without reducing ligand activity. Since the specific binding between the ligand and analyte is driven by a unique—and, in most cases, unknown—combination of physical forces, the regeneration conditions must be determined empirically.

Experience has shown that short pulses of 10–20 mM H₃PO₄ or 10 mM Glycine·HCl at pH 1.5–2.5 (part of Regeneration Scouting Kit 1, product code K RK1-50I) are often sufficient to achieve quantitative regeneration. However, some receptor-ligand pairs may require different conditions for successful regeneration. Occasionally, the interaction between two binding partners is so strong that binding becomes practically irreversible. In such cases, kinetic titration or capture immobilization of the ligand are promising strategies.

Andersson has proposed an innovative algorithm to streamline the otherwise time-consuming process of identifying optimal regeneration conditions [1][2]. His approach involves systematically combining six different regeneration cocktails. The composition of these cocktails, which XanTec distributes as the Regeneration Scouting Kit 2 (product code K RK2-50I), is outlined in the table below:

| Stock solution | Product code | Composition |
|---------------------------------|--------------|---|
| Acidic | B RCA-50ML | 37.5 mM Oxalic acid, 37.5 mM H ₃ PO ₄ , 37.5 mM formic acid, 37.5 mM malonic acid pH 5.0 |
| Alkaline | B RCB-50ML | 0.2 M Ethanolamine, 0.2 M Na ₃ PO ₄ , 0.2 M Piperazine, 0.2 M Glycine pH 9.0 |
| Chaotropic | B RCI-50ML | 0.46 M KSCN, 1.83 M MgCl ₂ , 0.92 M Urea, 1.83 M Guanidine·HCl |
| Non-polar, water-soluble | B RCS-50ML | 20 % (v/v) DMSO, 20 % (v/v) formamide, 20 % (v/v) ethanol, 20 % (v/v) acetonitrile, 20 % (v/v) 1-butanol |
| Detergent | B RCD-50ML | 0.3 % (w/v) CHAPS, 0.3 % (w/v) Zwittergent 3-12, 0.3 % (v/v) Tween 80, 0.3 % (v/v) Tween 20, 0.3 % (v/v) Triton X-100 |
| Chelating | B RCC-50ML | 0.02 M disodium EDTA |

Procedure for regeneration screening and optimization

- 1 Prepare the first regeneration cocktail by mixing one part of one of the six stock solutions with two parts of water.
- 2 Inject the analyte until equilibrium is reached.
- 3 **Screening:** Inject the first regeneration cocktail and measure its effect as a percentage of the analyte removed from the sensor chip. No effect corresponds to 0 % regeneration, while complete removal equals 100 % regeneration. If the regeneration efficiency is below 10 %, proceed to inject the next regeneration cocktail (1 part stock solution, 2 parts water). If the analyte level drops below 67 % of the original value, inject new analyte and re-saturate the surface. Repeat until all cocktails have been evaluated.

The regeneration efficacy R_e is calculated by the following formula:

$$R_e = (\text{analyte loss}) / (\text{analyte level}) \times 100 \%$$

- 4 **Optimization:** Identify the two to three regeneration cocktails with the highest R_e and recombine them using a 2D (two best regeneration cocktails) or 3D (three best regeneration cocktails) experimental mixture design. Add water if the new cocktails do not reach 100 % volume. Re-evaluate the R_e of the new regeneration solutions.
- 5 If regeneration remains insufficient, follow the trends observed in the previous optimization experiment and iterate until regeneration is satisfactory.

Note: Repeated short pulses of the regeneration solution are generally more effective than increasing the injection time.

STORAGE OF USED SENSOR CHIPS

The storage and reuse of XanTec's NeutrAvidin™ sensor chips are not recommended, as the captured ligand gradually leaches from the chip surface, reducing immobilization capacity and potentially causing cross-contamination of the sensor surface's reference areas.

TROUBLESHOOTING

| Issue | Possible solution |
|---|---|
| Insufficient ligand immobilization level | <p>Make sure that the NAP/ NAHLC sensor chip provides a sufficient immobilization capacity. Otherwise, switch to a chip variant that provides higher immobilization levels.</p> <p>Make sure that all interfering components of your ligand stock solution (such as free biotin) are completely removed before immobilization. Sometimes, multiple desalting steps are necessary for quantitative removal.</p> <p>Increase the biotin ligand contact time.</p> <p>Increase the concentration of your biotinylated ligand.</p> |
| Insufficient ligand activity | <p>Check ligand and analyte integrity in your stock solutions and in the immobilization buffer with regard to activity, aggregation, and biological contamination.</p> <p>Reduce the molar excess of the biotinylation reagent used during the ligand biotinylation process. If applicable, switch to a site-directed ligand biotinylation approach.</p> <p>If the ligand was purchased pre-biotinylated, inquire about the degree of biotinylation. Many commercially available formulations have biotinylation levels that are unsuitable for SPR applications.</p> <p>Decrease the overall immobilization level to minimize ligand crowding.</p> |
| Ligand leaching | <p>If high ligand immobilization levels are used and your SPR system employs a serial flow cell configuration, the biotinylated ligand may gradually leach from the immobilized channel, leading to cross-contamination of the reference channel. In such cases, it is recommended to block the reference channel with water-soluble biotin analogs and reduce the ligand immobilization levels.</p> |

LITERATURE

1. Andersson, K., Areskoug, D., & Hardenborg, E. (1999). Exploring buffer space for molecular interactions. *Journal of Molecular recognition*, 12(5), 310-315.
2. Andersson, K., Hämäläinen, M., & Malmqvist, M. (1999). Identification and optimization of regeneration conditions for affinity-based biosensor assays. A multivariate cocktail approach. *Analytical chemistry*, 71(13), 2475-2481.

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