CHLOROALKANE-MODIFIED SENSOR CHIPS (HO)

Instructions for use

PRODUCT DESCRIPTION

Product code	Prefix (designates the instrument): SCB, SCBS, SCBN, SPP, SCBI, SPSM, SCH, SPMX, SCR, SCS, SD + Add: HOP, HOD200M, HOHC200M Example: SCBS HOD200M
Intended purpose	Site-directed, specific, covalent immobilization of Halo-tagged® fusion proteins on chloroalkane-functionalized 2D (HOP) or 3D (HOD, HOHC) sensor chips under preconcentration conditions. The coupling procedure does not require modification of either the chip surface or the ligand, thus offering a convenient alternative to EDC/NHS coupling. Recommended applications include the investigation of biomolecular interactions involving proteins, nucleic acids, and small molecules.
Storage	Store at -20 °C, desiccated over molecular sieve 4A, or at 2–8 °C in physiological buffer.
Related products	• Borate elution buffer, product code B BELU-50ML • Coupling buffers (acetate or maleate) pHs 4.0–6.0, product codes B A40-50ML, B A45-50ML, B A50-50ML, B A55-50ML, and B M60-50ML

INTRODUCTION

HOP, HOHC, and HOD sensor chips are coated with either a bioinert 2D carboxylate (HOP) or a 3D polycarboxylate hydrogel (HOHC, HOD), each derivatized with short-chain chloroalkanes. These functional groups form stable covalent bonds with fusion proteins bearing a 33-kDa HaloTag®. The reaction is rapid, highly specific, and occurs spontaneously under preconcentration conditions, ensuring uniform ligand orientation on the sensor surface.

Compared to conventional non-selective methods such as EDC/NHS activation, the HaloTag® immobilization process offers substantial advantages. It is not only significantly more convenient but also allows for a more controlled and well-defined immobilization. By avoiding random ligand orientation and ligand crosslinking, which often occur with EDC/NHS coupling, this approach results in a homogeneous ligand population, ultimately leading to improved data quality and reproducibility in kinetic and affinity assays. Consequently, experimental workflows are streamlined, requiring fewer optimization steps, while the consistency and integrity of kinetic interaction analyses are enhanced, making it easier to draw reliable conclusions about binding kinetics and affinities.

Adequate electrostatic preconcentration remains essential for successful protein immobilization. However, traditional preconcentration scouting cannot be performed, as Halo-tagged fusion proteins would directly bind to the sensor chip. Therefore, preconcentration and immobilization must be evaluated simultaneously. For further details, please refer to the protocol below.

ADDITIONAL MATERIALS REQUIRED FOR HALOTAG® COUPLING

Recombinant Halo-tagged® fusion protein (to be provided by the user)

Coupling buffer (dependent on the pI of the ligand):
Acetate buffer pH 4.0 (product code B A40-50ML): 5 mM sodium acetate, pH 4.0, 50 mL
or Acetate buffer pH 4.5 (product code B A45-50ML): 5 mM sodium acetate, pH 4.5, 50 mL
or Acetate buffer pH 5.0 (product code B A50-50ML): 5 mM sodium acetate, pH 5.0, 50 mL
or Acetate buffer pH 5.5 (product code B A55-50ML): 5 mM sodium acetate, pH 5.5, 50 mL
or Maleate buffer pH 6.0 (product code B M60-50ML): 2.5 mM sodium maleate, pH 6.0, 50 mL

Borate elution buffer (product code: B BELU-50ML): 0.1 M sodium borate, 1 M NaCl pH 9.0, 50 mL

PREPARATIONS FOR HALOTAG® COUPLING

Clean the SPR fluidics

Make sure that the flow system of your SPR equipment is free from any protein contamination, because even minor amounts of desorbed protein will concentrate onto the charged sensor surface. If necessary, clean the system with either 1 % Tween 20 or – more stringent – 0.5 % SDS for 5 min followed by 50 mM glycine·HCl (pH 9.5) for 10 min (both part of the Desorb Kit, product code K D-500ML). The glycine is necessary to remove traces of SDS.

Ligand preparation

Dilute your Halo-tagged® protein in coupling buffer. Recommended concentrations are 1 to 100 µg/mL. The pH of the coupling buffer should be below the protein's isoelectric point to ensure a positive net charge of the ligand which is required for electrostatic interaction with the negatively charged chip surface. **Please note that preconcentrated Halo-tagged® protein instantly reacts with the surface bound chloroalkane and thus cannot be removed afterwards.**

Sensor chip

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

After opening the pouch, install the sensor chip according to 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 COUPLING OF HALO-TAGGED® PROTEINS

	Procedure	Flowrate [µL/min]	Injection time [s]
1	Equilibrate your SPR-system with water as running buffer and mount a XanTec HOP, HOHC or HOD sensor chip.		
2	Condition the surface with Borate elution buffer. Wait until the baseline has stabilized.	25	3 × 60
3	If preconcentration conditions are known, inject the Halo-tagged® protein solution (1–100 µg/mL) in a suitable Coupling buffer. Starting with low concentrations of 1–5 µg/mL is recommended to avoid excessive ligand immobilization. Adjust injection time and protein concentration as required to achieve your target immobilization level. Wait until the baseline has stabilized and continue with step 5.	10	600
4	Optional: If preconcentration conditions are unknown, Inject your Halo-tagged® protein at low concentrations (1–5 µg/mL) in Maleate Coupling Buffer pH 6.0. If you can detect a continuously increasing binding signal, protein preconcentration takes place. Continue with injecting your Halo-tagged® protein until the desired immobilization level has been reached. Adjust injection time and protein concentration as required to achieve your target immobilization level. If no steady signal increase during protein injection occurs, switch to the next coupling buffer 0.5 pH below the previous one. Repeat this step until a sufficient preconcentration of the protein is observed on the sensor surface.	10	300
5	Inject Borate elution buffer.	25	60
6	Switch to a Tween-containing physiological running buffer, such as HBSTE or HBSTE+, and wait until the baseline stabilizes.		
7	Start interaction analysis. We recommend beginning with 3–5 consecutive regeneration cycles to improve data quality and stabilize the chip surface.		

Notes

It is recommended to start with low ligand concentrations and re-immobilize with higher concentrations as needed. This approach prevents excessive immobilization on the sensor coating, which is a common cause of poor experimental data quality in kinetic analyses. Additional immobilization steps can also be conducted during the interaction experiment.

A physiological running buffer can be used instead of water during immobilization.

If a non-functionalized (poly)carboxylate sensor chip is available, preconcentration scouting can be conducted on this sensor chip to evaluate ideal immobilization conditions in advance.

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 for storage.

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 H_3PO_4 or 10 mM glycine·HCl at pH 1.5–2.5 (part of Regeneration Scouting Kit 1, product code K RK1-50l) 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 [2][3]. 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 RK2-50l), is outlined in the table below:

Stock solution	Product code	Composition
Acidic	B RCA-50ML	37.5 mM Oxalic acid, 37.5 mM H_3PO_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_3PO_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= (analyte loss)/(analyte level) × 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

For later reuse, sensor chips can be stored either dry or wet under physiological conditions. When handling the sensor chip, avoid touching the top coating with gloves or tweezers.

Biacore users only: To prevent detachment of the glass chip in the instrument after chips have been stored under buffer or at 100 % humidity, we strongly recommend checking the mechanical stability of the assembly before inserting the chip cartridge into the instrument.

Reichert users only: If the sensor chip is intended for later reuse, use the refractive index matching foil instead of immersion oil when installing the sensor chip for the first time. Oil traces may contaminate the hydrogel top coating after chip removal, potentially causing irreversible damage to the immobilized ligand.

Dry storage

1. Dismount the used sensor chip from your SPR instrument.
2. Rinse the hydrogel surface of the sensor chip carefully with ultrapure water.
3. Optional: Carefully remove excess water from the edge of the hydrogel coating using a pipette. Place a droplet (30 µL for SCB and SCBS) of XanTec stabilization buffer onto the wet chip surface and allow it to spread, ensuring it covers the entire surface. Let it dry for approximately 60 minutes in a desiccator with desiccant (4A molecular sieve). This step helps prevent denaturation of the immobilized ligand and prolongs the shelf-life of the sensor chip.
4. Dry the sensor chip with a jet of filtered air or nitrogen.
5. Store the sensor chip dry, using a 3A or 4A molecular sieve, in a cold environment (-25 °C) under an inert gas atmosphere in a tightly sealed container. The stability of the sensor chip depends on the stability of the immobilized ligand. The underlying hydrogel coating should remain stable for several weeks to months.
6. **Reinstallation**

   No protective top coating: Equilibrate the sensor chip to room temperature before opening the storage container, then insert the chip according to the instrument manufacturer's instructions.

   Protective top coating applied: Equilibrate the sensor chip to room temperature before opening the storage container. Immerse the chip in physiological buffer for 10 minutes to remove the protective layer from the hydrogel coating. Rinse gently with ultra-pure water and carefully dry it using a stream of filtered air or nitrogen.

Wet storage

1. Dismount the used sensor chip from your SPR instrument.
2. Rinse the hydrogel surface of the sensor chip carefully with ultra-pure water. Place the sensor chip in a container filled with sterile filtered, physiological buffer and seal it tightly. For Cytiva sensor chips, 50 mL centrifugation tubes are applicable. Store the sensor chip refrigerated at 2–8 °C.

   The stability of the sensor coating mainly depends on the stability of the immobilized ligand. The underlying hydrogel coating should be stable for several days to weeks at such conditions. Long-term storage in water is not advised, as this can negatively affect the integrity of the sensor coating
3. **Reinstallation**

   Remove the sensor chip from the container, preferably using clean tweezers. Rinse with ultra-pure water to remove buffer salts, and carefully dry it using a stream of filtered air or nitrogen. Then, insert the chip according to the instrument manufacturer's instructions.

TROUBLESHOOTING

Issue	Possible solution
Insufficient electrostatic preconcentration	Perform electrostatic preconcentration scouting to check for optimal preconcentration conditions, ideally on a nonderivatized (poly)carboxylate sensor chip of the same type (e. g. CMD200M when using HOD200M). Desalt protein directly into Coupling buffer to remove possible salt contaminants. Lower the ionic strength of the coupling buffer. The ligand is too acidic (pI < 3.0) and does not preconcentrate on polycarboxylate sensor chips. In this case, alternative coupling methods should be considered.
Insufficient protein immobilization level	Make sure that optimal electrostatic preconcentration conditions were chosen. Increase the protein concentration. Increase the protein contact time. Decrease the ionic strength of the immobilization buffer. Switch to another chip type with a higher immobilization capacity.
High nonspecific binding	Use a physiological running buffer with Tween like HBSEP or HBSEP+ Add BSA to your running buffer with concentrations of up to 3 %. Increase the salt concentration of the running buffer.

LITERATURE

1. *Los, G. V., Encell, L. P., McDougall, M. G., Hartzell, D. D., Karassina, N., Zimprich, C., ... & Wood, K. V. (2008). HaloTag: a novel protein labeling technology for cell imaging and protein analysis. ACS chemical biology, 3(6), 373-382.*
2. *Andersson, K., Areskoug, D., & Hardenborg, E. (1999). Exploring buffer space for molecular interactions. Journal of Molecular recognition, 12(5), 310-315.*
3. *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.*

*For **in-vitro** use only. Not for use in clinical diagnostic procedures.*

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