Protein Production & Assays

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Protocol

Twin-Strep-tag®
Capture Kit for SPR

1 GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Kit components:

	Twin-Strep-tag® Capture Kit (Cat. No. 2-4370-000)	Twin-Strep-tag® Capture Maxi Kit (Cat. No. 2-4370-010)
SPR Immobilization Buffer (10 mM Sodium Acetate, pH 4.5)	3 ml	30 ml
Regeneration Buffer (3 M GuHCl)	20 ml	200 ml
Strep-Tactin®XT (53 kDa)	100 µl (1 mg/ml in solution)	1 mg (lyophilized)
GFP-Twin-Strep-tag (control protein, 30 kDa)	30 µg (lyophilized)	30 µg (lyophilized)

Required reagents:

- Amine Coupling Kit (Cat. No. BR-1000-50, Cytiva)
- Carboxyl-derivatized sensor chip (e.g. Sensor Chip CM5, Cytiva)
- Running Buffer, e.g. HBS-EP (10 mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05% Surfactant P20), HBS-P, HBS-N, or PBS
- 1x Buffer W (100 mM Tris-Cl, 150 mM NaCl, 1 mM EDTA) for solubilization of GFP-Twin-Strep-tag

Storage: Store all kit components at 2 – 8 °C and GFP-Twin-Strep-tag at -20 °C after solubilization.

Stability: All products are stable for 12 months after shipping.

Shipping: Room temperature

Warnings: Warnings are stated on the Material Safety Data Sheet.

Important information: The Twin-Strep-tag® Capture Kit and Twin-Strep-tag® Capture Maxi Kit is intended for site-directed, reversible capture of Twin-Strep-tag® proteins for biomolecular interaction analysis using Biacore™ SPR systems. It is highly recommended to use Twin-Strep-tag® in place of Strep-tag®II for this approach, since the higher affinity of the Twin-Strep-tag® to Strep-Tactin®XT leads to long-term stable binding on the chip surface.

2 Description

The Strep-tag® technology is one of the most widely used affinity chromatography systems and it allows, in addition to purification, the detection and immobilization of recombinant proteins as well. Constant developments lead to a powerful tool, which is based on the Strep-Tactin®XT in combination with the Twin-Strep-tag® (WSHPQFEK-GGGSGGGSGG-SA-WSHPQFEK), the tandem Strep-tag®II. Strep-Tactin®XT has a binding affinity in low pM range for the Twin-Strep-tag®. This high affinity enables new applications in the field of high throughput screening and analytic applications, making the technology superior to all other available affinity tag systems. One of these applications is SPR (surface plasmon resonance), which is addressed with the Twin-Strep-tag® Capture Kit and the larger variant, Twin-Strep-tag® Capture Maxi Kit. Thereby, Strep-Tactin®XT (capture molecule) is immobilized onto the surface of a SPR sensor chip, e.g. Biacore™ CM5, which then efficiently captures Twin-Strep-tag® proteins (ligand) whereby binding affinities and/or kinetics to a specific analyte can be determined. Therefore, the analyte can be present in culture supernatant, cell extracts, or various buffers.

3 INITIAL PREPARATIONS

3.1 Dissolve GFP-Twin-Strep-tag in 100 µl 1x Buffer W to obtain a 10 µM solution.

3.2 In case of the Twin-Strep-tag® Capture Maxi Kit, 1 mg Strep-Tactin®XT is lyophilized from 25 mg/ml solution pH 7.4 containing 4 mM KH_2PO_4, 16 mM Na_2HPO_4, 115 mM NaCl, 1 mM EDTA. Dissolve Strep-Tactin®XT in 1 ml water to obtain a 1 mg/ml solution (18.87 µM). Strep-Tactin®XT of the Twin-Strep-tag® Capture Kit is already in solution and provided in the same buffer conditions.

3.3 Reagents for immobilization are provided in the Amine Coupling Kit (Cytiva, BR-1000-50). Prepare all buffers according to the Amine Coupling Kit protocol.

3 PROTOCOL

3.1 Immobilization of Strep-Tactin®XT on CM5 chip

- Perform all steps at 25 °C and using a flow rate of 10 µl/min.
- It is not recommended to use an unmodified surface as reference. The reference surface should be prepared in the same way using the same settings as the active surface.

3.1.1 Prepare Strep-Tactin®XT solution with a concentration of 20 – 50 µg/ml for high density surfaces or 5 – 10 µg/ml for low density surfaces in SPR Immobilization Buffer.

3.1.2 Pre-treat the chip surface with 50 mM NaOH (three consecutive 1-minute pulse injections).

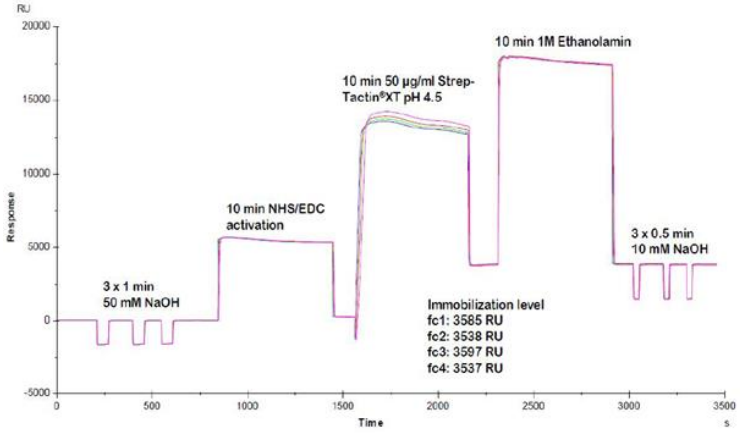
3.1.3 Activate the chip by using freshly prepared EDC/NHS mixture for 10 min.

3.1.4 Couple Strep-Tactin®XT by running Strep-Tactin®XT solution for 10 min.

3.1.5 Wash with running buffer until baseline is stable.

3.1.6 Deactivate with Ethanolamine for 10 min.

3.1.7 Perform surface conditioning with 10 mM NaOH (three consecutive 30-second pulse injections).



The sensorgram, produced with Biacore™ T200 (Cytiva), shows a typical immobilization sequence of Strep-Tactin®XT on sensor chip CM5 with four flow cells simultaneously. Conditions and obtained response units (RU) were as indicated. The procedure results in immobilization levels > 3300 RU. At these levels, the exact amount of immobilized Strep-Tactin®XT is normally not critical for capturing Twin-Strep-tag® proteins. The immobilization level may be adjusted, if necessary, by modulating the contact time or concentration of Strep-Tactin®XT. A high density of Strep-Tactin®XT on a chip surface ensures a stable baseline after capture of Twin-Strep-tag® protein.

3.2 Capture of Twin-Strep-tag® protein on Strep-Tactin®XT coated chip and kinetic measurement

- Start-up cycles: For best assay performance, run at least one start-up cycle using identical settings as for the analysis cycles, including Twin-Strep-tag® protein and buffer instead of analyte.
- Capture conditions of the Twin-Strep-tag® protein depend on the concentration and binding characteristics of it and the purpose of the experiment.
- Biotin, a common ingredient of cell culture media or present in cell extracts, has to be masked by avidin or BioLock in analyte samples. Otherwise, it could elute the captured Twin-Strep-tag® protein during the sample injection. A detailed protocol for biotin blocking can be downloaded at www.iba-lifesciences.com/download-area-protein.html.

3.2.1 Typical conditions for the capture of Twin-Strep-tag® proteins are a concentration of 50 nM and a contact time of 1 – 2 min. For kinetic sample measurements, the capture level of the Twin-Strep-tag® protein has to be calculated according to the molecular masses of captured ligand and analyte sample to obtain a final R_{max} of 20 – 100 RU for the interacting sample on the captured ligand. Low level capture of ligand can be performed with lower concentrations of Twin-Strep-tag® protein and shorter contact times.

3.2.2 Inject the analyte containing sample. Choose injection conditions appropriate to the assay purpose. For kinetic sample measurements, the concentrations of the analyte should be ten times higher and lower as the expected K_D. For example, if the K_D is 1 nM the concentrations should be in a range from 0.1 – 10 nM. If the K_D is not known, test experiments with widely spaced concentrations (at least five concentrations from pM to nM) are necessary to give a first indication.

3.3 Regeneration of Strep-Tactin®XT coated chip

3.3.1 Regenerate chip by application of Regeneration Buffer (3 M GuHCl) with three consecutive 1-minute injections until baseline is stable.

3.3.2 Remove Regeneration Buffer by injection of Running Buffer until baseline is stable. After that, Strep-Tactin®XT is still bound to the SPR chip and the next sample can be injected.

4 TROUBLESHOOTING

Inefficient regeneration	In cases, where unsatisfying regeneration results are obtained, it might worth trying 10 mM NaOH with 500 mM NaCl (freshly prepared) or 3 M $MgCl_2$ as alternative. Furthermore, the addition of 2% Dioxan or 0.1 – 0.25% SDS to the regeneration solutions can be tested but avoid acidic regeneration procedures.
Inefficient coupling due to incorrect Strep-Tactin®XT concentration	The optimal protein concentration is 20 – 50 µg/ml for high density surfaces and 5 – 10 µg/ml for low density surfaces. Concentrations > 50 µg/ml will increase the ionic strength and reduce coupling efficiency.
Inefficient coupling due to incorrect pH	The optimal pH range is 4 – 5 and a pH of 4.5 is recommended, whereas a pH value > 5 does not work.

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EP Patent Application No. 1370574

B) STREPTAVIDIN MUTEINS AND METHODS OF USING THEM
US Patent No. 10,065,996
EP Patent No. 2 920 204
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