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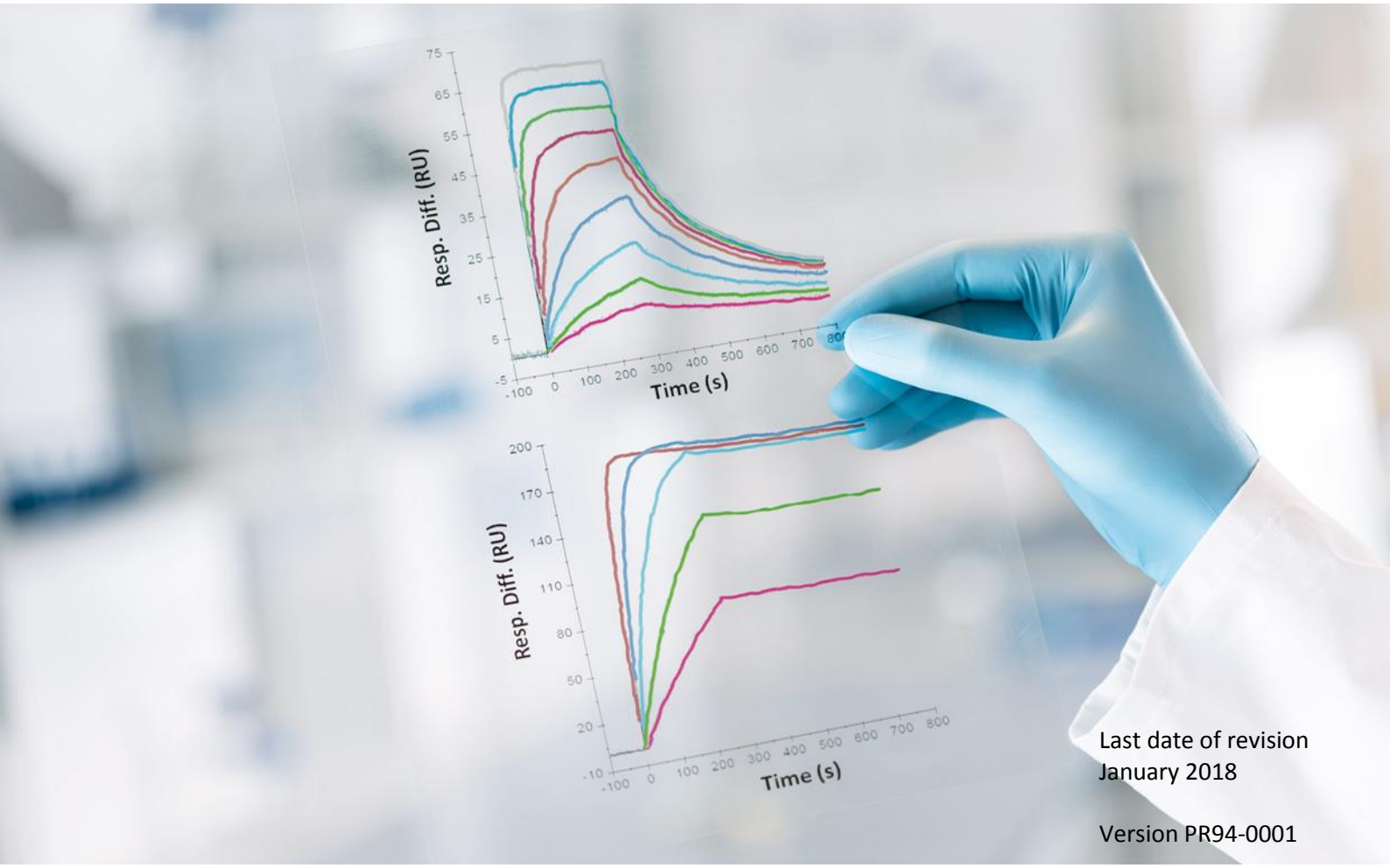
Protein Production & Assays

Twin-Strep-tag® Capture Kit for SPR

Manual



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Content

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1 Introduction

Strep-tag® system

The Strep-tag®:Strep-Tactin® system is one of the most widely used affinity chromatography systems and it allows purification, detection and immobilization of recombinant proteins.
Constant developments lead to the 3rd generation Strep-tag® system, which is based on the novel **Strep-Tactin®XT** in combination with Twin-Strep-tag®, the tandem Strep-tag®II (**WSHPQFEK**-GGGSGGGSGG-SA-**WSHPQFEK**).
Strep-Tactin®XT has a **binding affinity in low pM range** for the Twin-Strep-tag® still maintaining the binding reversibility, the mild recovery of immobilized proteins and the high purity (> 95 %). This high affinity of Strep-Tactin®XT enables new applications in the field of high throughput screening, batch purification, purification under denaturing conditions and protein interaction analysis, making the system superior to all other available affinity tag purification systems.

Furthermore the extremely high binding affinity can be used in assays like SPR (surface plasmon resonance) to study biomolecular interactions. For this application **Strep-Tactin®XT** (the capture molecule) is immobilized onto the surface of the SPR sensor chip e.g. Biacore CM5. Subsequently, the Twin-Strep-tag® fusion protein (the ligand), is then efficiently captured on the **Strep-Tactin®XT** coated chip whereby binding affinities and/or kinetics can be determined.
It is strongly recommended to use Strep-Tactin®XT in combination with Twin-Strep-tag® for this approach, as the higher affinity of Twin-Strep-tag® to Strep-Tactin®XT in pM ranges leads to long-term stable binding on the chip surface.

The **Twin-Strep-tag® Capture Kit** is intended for site-directed, reversible capture of Twin-Strep-tag® fusion proteins for biomolecular interaction analysis using Biacore™ SPR systems. The Kit consists of Strep-Tactin®XT, a Control Protein, Immobilization- and Regeneration buffer and has to be used in combination with the Amine Coupling Kit (GE Healthcare, see page 5).

2 Immobilization of Strep-Tactin®XT on Biacore sensor chips

Kit content:

Buffers/Solutions	Concentration of ingredients
Strep-Tactin®XT; 100 µl	1 mg/ml in PBS, 1 mM EDTA
Immobilization buffer; 3 ml	10 mM Sodium acetate, pH 4.5
Regeneration buffer; 20 ml	3 M GuHCl
Control protein; 30 µg	GFP-Twin-Strep-tag®, lyophilized

Additional products required:

Amine Coupling Kit	GE Healthcare; BR100050
Carboxyl-derivatized sensor chip	GE Healthcare; e.g. Sensor Chip CM5
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, PBS

2.1 Immobilization of Strep-Tactin®XT on CM5 chip

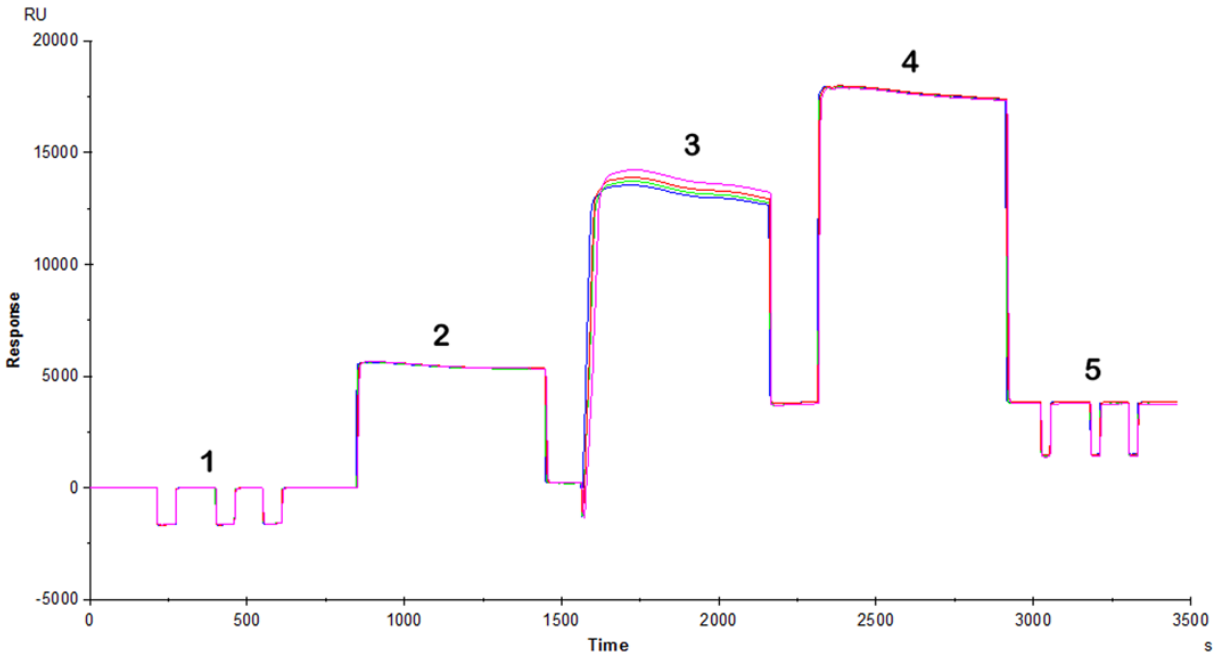
Important Note	1. Perform all steps at 25°C and using a flow rate of 10 µl/min. 2. It is not recommended to use an unmodified surface as reference. The reference surface should be prepared in the same way using the same settings as the active surface.

Protocol	**1. Prepare Strep-Tactin®XT solution with a concentration of 50 µg/ml in immobilization buffer (5 µl Strep-Tactin®XT + 95 µl Immobilization buffer).** **2. Reagents for immobilization are provided in the Amine Coupling Kit (GE Healthcare; BR100050).** **3. Pretreat the chip surface with 50 mM NaOH with 3 consecutive pulse injections of 1 min.** **4. Activate the chip by using freshly prepared EDC/NHS mixture for 10 min.** **5. Couple Strep-Tactin®XT by running Strep-Tactin®XT solution for 10 min.**

6. **Wash with ultrapure water for 30 min or until baseline is stable.**

7. **Run deactivation buffer with Ethanolamine for 10 min.**

8. **Perform surface conditioning with 10 mM NaOH with 3 consecutive pulse injections of 30 sec.**



The sensorgram shows a typical immobilization sequence for Strep-Tactin®XT on Sensor Chip CM5 for 4 flow cells simultaneously. The numbers indicate the injections of 3x 50 mM NaOH (1), EDC/NHS (2), Strep-Tactin®XT (3), ethanolamine (4) and 3x 10 mM NaOH (5).

This procedure should result in immobilization levels of 3300 RU or more on Sensor Chip CM5. At these levels, the exact amount of immobilized Strep-Tactin®XT is normally not critical for capturing Twin-Strep-tag® fusion proteins. The immobilization level may be adjusted if necessary by adjusting 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® fusion protein.

2.2 Ligand injection - capturing of a Twin-Strep-tag® fusion protein (ligand) on the Strep-Tactin®XT coated chip and regeneration

Required buffer	
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, PBS

Important Note

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® fusion protein ligand and buffer instead of analyte.

Ligand injection: Twin-Strep-tag® fusion protein ligand capturing conditions depend on the concentration and binding characteristics of the ligand and the purpose of the experiment.

Protocol

1. **Typical conditions: Twin-Strep-tag® fusion ligand with a concentration of 50 nM and a contact time of 1-2 minutes.**
   For kinetic sample measurements the capture level for Twin-Strep-tag® fusion proteins 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® fusion protein and shorter contact times.

2. **Inject the sample.**
   Use sample injection conditions appropriate to the assay purpose.
   Note: The sample must not contain biotin which elutes the Twin-Strep-tag® protein.

3. **For regeneration of the chip use Regeneration buffer (3 M GuHCl) with 3 consecutive 1-minute injections until baseline is stable.**
   In cases where unsatisfying regeneration results are obtained it might Worth trying 10 mM NaOH/ 500 mM NaCl (freshly prepared) or 3 M $MgCl_2$ as alternative.

4. **Inject Running buffer to remove GuHCl until baseline is stable.**
   Note: After the regeneration, Strep-Tactin®XT is still bound to the SPR chip and the next sample can be injected.

Important Note

For proteins where these conditions do not give adequate regeneration, a combination of solutions or an addition of 2 % Dioxan to the regeneration solutions may be tested. Avoid acidic regeneration procedures, but 0.1 - 0.25 % SDS may also be tested.

3 Troubleshooting

Strep-Tactin®XT coupling efficiency too high or too low	
Problem	**Suggestion**
- Protein concentration (Strep-Tactin®XT)	Optimal protein concentration: 20-50 µg/ml for high density surfaces, 5-10 µg/ml for low density surfaces. **Concentrations > 50 µg/ml will increase the ionic strength and reduce coupling efficiency.**
- pH	Optimal pH range 4-5, 4.5 is recommended whereas > pH 5.0 does not work
- Regeneration efficiency	Best regeneration efficiency is observed with 3 M GuHCl. If alternative buffers are required 3 M $MgCl_2$ or 10 mM NaOH (freshly prepared) might also be used/tested.

Please download always an up-to-date version of this protocol from:
www.iba-lifesciences.com/download-area.html.

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