

**! For research use only**

Protocol

# Twin-Strep-tag<sup>®</sup> Capture Kit for SPR

## 1 GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Kit components:

	Twin-Strep-tag <sup>®</sup> Capture Kit (Cat. No. 2-4370-000)	Twin-Strep-tag <sup>®</sup> 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 <sup>®</sup> 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<sup>®</sup> Capture Kit and Twin-Strep-tag<sup>®</sup> Capture Maxi Kit is intended for site-directed, reversible capture of Twin-Strep-tag<sup>®</sup> proteins for biomolecular interaction analysis using Biacore<sup>™</sup> SPR systems. It is highly recommended to use Twin-Strep-tag<sup>®</sup> in place of Strep-tag<sup>®</sup>II for this approach, since the higher affinity of the Twin-Strep-tag<sup>®</sup> to Strep-Tactin<sup>®</sup>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<sub>2</sub>PO<sub>4</sub>, 16 mM Na<sub>2</sub>HPO<sub>4</sub>, 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.

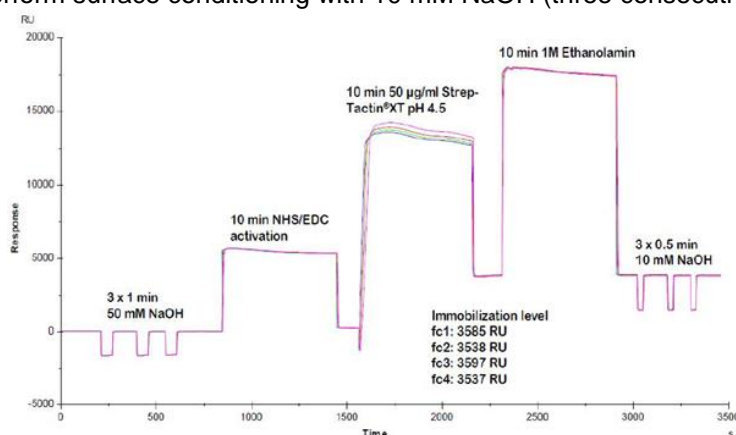
## 3 PROTOCOL

### 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](http://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

<b>Inefficient regeneration</b>	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 <sub>2</sub> 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.
<b>Inefficient coupling due to incorrect Strep-Tactin®XT concentration</b>	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.
<b>Inefficient coupling due to incorrect pH</b>	The optimal pH range is 4-5 and a pH of 4.5 is recommended, whereas a pH value > 5 does not work.

# End User Limited Use License

By and between  
IBA Lifesciences GmbH  
Göttingen  
("SELLER")

and  
Purchaser of  
Licensed Protein (as defined below)  
("PURCHASER ")

NOTICE: PURCHASE OF THIS PRODUCT IS ALSO SUBJECT TO IBA Lifesciences GmbH TERMS AND CONDITIONS OF SALE. A COMPLETE COPY OF SUCH TERMS AND CONDITIONS ARE FOUND AT <https://www.iba-lifesciences.com/terms-and-conditions.html> IN THE EVENT THAT ANY TERMS OR CONDITIONS OF THIS END USER LIMITED USE LICENSE (the "LIMITED USE LICENSE") AND IBA GmbH's TERMS AND CONDITIONS OF SALE CONFLICT OR IN CASE OF ANY DOUBT, THE MOST RESTRICTIVE TERMS AND CONDITIONS SHALL APPLY TOWARDS THE PURCHASER.

The compound(s) you have purchased (the "Licensed Protein", as specifically defined below), and/or the use thereof, may be covered by claims in the following patents controlled by IBA Lifesciences GmbH (collectively "Patent Rights"):

## PATENT RIGHTS

A) SEQUENTIALLY ARRANGED STREPTAVIDIN-BINDING MODULES AS AFFINITY TAGS  
US Patent No. 7,981,632  
US Patent No. 8,735,540  
DE Patent No. 101 13 776  
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  
JP Patent No. 6475630  
CN Patent No. 105073770  
Pending patent applications in US, CA

## 1. Definitions.

Whenever used in this Limited Use License document with an initial capital letter, the terms defined below, whether used in the singular or the plural, shall have the meanings specified below.

1.1 "Commercial Purpose" means any activity conducted in exchange for consideration including, but not limited to, (a) use of the Tag in research and development activities of a commercial entity or in manufacturing, (b) use of the Tag to provide a service, information or data, other than to perform Contract Research (as defined below), (c) use of the Tag for therapeutic, diagnostic or prophylactic purposes and (d) sale of the Tag, whether or not such Tag is resold for use in research. As used herein, "Contract Research" means surface plasmon resonance (SPR) spectroscopy services performed by a company on a fee-for-service basis wherein said company receives the Tag from their customers and wherein said company uses said Tag within surface plasmon resonance (SPR) spectroscopy only.

1.2. "Licensed Protein" shall mean Strep-Tactin XT as described in and protected by the Patent Rights (B), the use of which but for this Limited Use License would infringe one or more Valid Claims of Patent Rights (B).

1.3. "Tag" shall mean a protein or any other molecule comprising the amino acid sequence SAWSHQPFEKGGGGGGGGSAWSHQPFEK described in and protected by the Patent Rights (A), the use of which but for this Limited Use License would infringe one or more Valid Claims of Patent Rights (A).

1.4. "Field" shall mean immobilization of Licensed Protein in close proximity to a gold layer on a solid support (e.g. a chip) that is dedicated to enable surface plasmon resonance (SPR) spectroscopy and using said solid support for the binding of molecules comprising the Tag.

1.5. "Valid Claim" means: (a) a claim of an issued and unexpired patent within the Patent Rights that has not been (i) held permanently revoked, unenforceable, unpatentable or invalid by a decision of a court or governmental body of competent jurisdiction, unappealable or unappealed within the time allowed for appeal, (ii) rendered unenforceable through disclaimer or otherwise, (iii) abandoned, or (iv) lost through an interference proceeding; or (b) a pending claim of a pending patent application within the Patent Rights that (i) has been asserted and continues to be prosecuted in good faith and (ii) has not been abandoned or finally rejected without the possibility of appeal or refiling.

## 2. Limited Use License.

This Limited Use License assumes and is valid only if you acquired Licensed Protein from IBA Lifesciences. By purchasing Licensed Protein, you explicitly agree with the terms and conditions of this Limited Use License.

This Limited Use License grants to the Purchaser the non-transferable right to use the amount of Licensed Protein purchased by the Purchaser limited to the Field. By purchasing Licensed Protein, Purchaser agrees that (a) Purchaser shall not sell or otherwise transfer Licensed Protein to any other party, (b) Purchaser shall not use the amount of Licensed Protein purchased by such Purchaser beyond the Field, (c) Purchaser shall use Licensed Protein for Commercial Purposes only after having also received an appropriate license for the Tag from Seller (whereas, for sake of clarity, Seller in its own discretion may decide, in particular, (i) whether to grant such license to Purchaser or not, (ii) on the terms and conditions of such license as well as (iii) with respect to any consideration for such license grant), (d) Purchaser shall use Licensed Protein as well as the Tag in compliance with all applicable license terms and conditions, laws and regulations, including, without limitation, applicable human health and animal welfare laws and regulations, (e) Purchaser may transfer information or materials made through the use of Licensed Protein and Tag to a scientific collaborator only if such transfer is not for Commercial Purposes, and that such collaborator agrees in writing not to transfer such materials to any third party and to use such transferred materials and/or information solely for internal, non-clinical research and not for Commercial Purposes and (f) Licensed Protein and Tag have not been approved for use in humans by the U.S. Food and Drug Administration or any other regulatory body and may not be used in humans.

## 3. WARRANTY AND LIABILITY.

EXCEPT FOR FRAUDULENT INTENT (*ARGLIST*), AND/OR WHERE WE HAVE EXPLICITLY ACCEPTED A GUARANTEE FOR THEIR STATE, (*BESCHAFFENHEITSGARANTIE*), THE LICENSED PROTEIN AND THE PATENT RIGHTS ARE PROVIDED AS-IS AND WITH NO WARRANTY, EITHER EXPRESS OR IMPLIED, AND SPECIFICALLY WITHOUT ANY WARRANTY OF MERCHANTABILITY, NON-INFRINGEMENT OR FITNESS FOR A PARTICULAR PURPOSE. EXCEPT FOR LIABILITY FOR INTENTION (*VORSATZ*) AND TO THE EXTENT OTHERWISE PERMITTED BY LAW, UNDER NO CIRCUMSTANCES SHALL SELLER BE LIABLE FOR ANY CLAIMS, DEMANDS, LIABILITIES, COSTS, LOSSES, DAMAGES OR EXPENSES (INCLUDING LEGAL COSTS AND ATTORNEY'S FEES) OF WHATEVER KIND OR NATURE CAUSED TO OR SUFFERED BY ANY PERSON OR ENTITY THAT DIRECTLY OR INDIRECTLY ARISE OUT OF OR RESULT FROM OR ARE ENCOUNTERED IN CONNECTION WITH THE USE OF THE LICENSED PROTEIN OR IN THE FIELD. PURCHASER SHALL INDEMNIFY AND HOLD SELLER HARMLESS FROM AND AGAINST ANY AND ALL CLAIMS, DAMAGES, COSTS, EXPENSES AND OTHER LIABILITIES WITH RESPECT TO THE LICENSED PRODUCTS, FIELD AND THE PATENT RIGHTS.

## 4. Applicable Laws and Legal Venue.

This Label License is subject to and governed by the laws of Germany excluding the international conflict of law provisions. Exclusive legal venue shall be the courts being competent for the business seat of the Seller.



Check our Downloads page

[www.iba-lifesciences.com/download-area.html](http://www.iba-lifesciences.com/download-area.html)

for the latest version of this manual.



Info on warranty / licensing and trademarks available at:

[www.iba-lifesciences.com/patents-licenses-trademarks.html](http://www.iba-lifesciences.com/patents-licenses-trademarks.html)



If you have any questions, please contact

[strep-tag@iba-lifesciences.com](mailto:strep-tag@iba-lifesciences.com)

We are here to help!

