For research use only

Protocol

Strep-Tactin®XT BLI Coupling Kit

1 GENERAL INFORMATION & TECHNICAL SPECIFICATIONS

Cat. No.: 2-4380-000

Kit components:

Strep-Tactin®XT (53 kDa)	1 mg, lyophilized
EDC	100 mg
s-NHS	57.3 mg
Ethanolamine	20 ml (1 M ethanolamine, pH 8.5)
Immobilization Buffer	30 ml (10 mM sodium acetate, pH 4.5)
10x Buffer W	10 ml (1 M Tris-Cl, 1.5 M NaCl, 10 mM EDTA, pH 8)
Regeneration Buffer	20 ml (3 M GuHCl)

Required material and reagents:

- Amine reactive 2G biosensors (AR2G), Cat. No. 18-5092, Sartorius
- 15% w/v Saccharose for drying of Strep-Tactin®XT biosensors

Storage: **Store EDC and lyophilized Strep-Tactin®XT at -20 °C.** Store all other kit components at 2-8 °C.

Stability: All products are stable for 6 months after shipping.

Shipping: Cooled with blue ice

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

Important information: The Strep-Tactin®XT BLI Coupling Kit is intended for site-directed, reversible capture of Twin-Strep-tag® proteins for biomolecular interaction analysis using Bio-Layer Interferometry (BLI) devices. It is highly recommended to use Twin-Strep-tag® instead 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 biosensor surface. The kit components are sufficient for coating of one tray consisting of 96 biosensors. The protocol is adjusted for the Octet® instrument and describes coating and measurement with eight biosensors in parallel but can be easily adjusted for one biosensor in combination with the BLItz® instrument.

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. Constant developments of the technology 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 like Bio-Layer Interferometry (BLI), making the technology superior to all other available affinity tag systems. The Strep-Tactin®XT BLI Coupling Kit kit provides all necessary reagents for coating of up to 96 biosensors (one tray) with Strep-Tactin®XT and subsequent capture of Twin-Strep-tag® proteins (ligand) whereby binding affinities and/or kinetics to a specific analyte can be determined. For the measurements, the analyte can be present in culture supernatant, cell extract, or various buffers.

3 INITIAL PREPARATIONS

3.1 Strep-Tactin®XT stock solution

Dissolve Strep-Tactin®XT in 1 ml of sterile PBS to obtain a 1 mg/ml solution (18.87 µM). Store Strep-Tactin®XT solution at at 2-8 °C until needed. The solution is stable for 12 months.

3.2 Buffer W working solution

Dilute 1 volume of 10x Buffer W with 9 volumes of water to obtain 1x Buffer W. Store at 2-8 °C until needed.

3.3 EDC and s-NHS stock solution

For preparation of stock solutions, it is recommended dissolving each reagent in water and splitting each solution into aliquots of 100 µl. Aliquots of 100 µl will provide enough reagent to perform 8 immobilizations as described in the protocol below.

3.3.1 Dissolve EDC in 1300 µl of water to generate a 400 mM stock.

3.3.2 Mix to ensure complete dissolution of the solid.

3.3.3 Aliquot and freeze immediately at -20 °C. Store there until needed.

3.3.4 Dissolve s-NHS in 1320 µl of water to generate a 200 mM stock.

3.3.5 Mix to ensure complete dissolution of the solid.

3.3.6 Aliquot and freeze immediately at -20 °C. Store there until needed.

Reagent integrity will be maintained for at least 6 months under proper storage conditions. For each experiment, thaw one aliquot of EDC and one of s-NHS. Vortex briefly after thawing. Do not refreeze EDC and s-NHS aliquots. Use EDC and s-NHS within 15 minutes after mixing them together.

4 PROTOCOL

4.1 Hydration of biosensors

- Hydrate the required number of biosensors in water in a separate hydration plate at room temperature.

4.1.1 Pipet 200 µl water per well into a 96-well plate.

4.1.2 Insert the hydration plate into the biosensor tray.

4.1.3 Align the biosensor rack over the hydration plate and lower the biosensors into the wells, taking care to not scrape or touch the bottom of the biosensors.

4.1.4 Hydrate the biosensors for at least 45 minutes at room temperature.

4.2 Immobilization of Strep-Tactin®XT and subsequent drying of the biosensors for later use

- Equilibrate reagents and samples (except EDC and s-NHS aliquots) to room temperature prior to preparation. For frozen samples, thaw and mix thoroughly prior to use and chill on ice until needed.
- Use EDC and s-NHS within 15 minutes of mixing them together!
- Ensure that the Octet® instrument is turned on and the lamp is warmed up to room temperature for at least 40 minutes prior to starting the assay.

4.2.1 Pre-set the assay as indicated in table 1 in the data acquisition software.

4.2.2 Prepare a 96-well plate for immobilization and drying as shown in figure 1.

4.2.3 Pipet 200 µl/well water into column 1 and 5 of a 96-well plate.

4.2.4 Pipet 200 µl/well 1 M Ethanolamine pH 8.5 into column 4.

4.2.5 Pipet 200 µl/well 15% w/v Saccharose in column 6.

4.2.6 Prepare the required volume of Strep-Tactin®XT working solution. Therefore, dilute Strep-Tactin®XT with immobilization buffer to obtain a concentration of 50 µg/ml. Pipet 200 µl/well Strep-Tactin®XT working solution into column 3.

4.2.7 Prepare 2000 µl of an EDC/s-NHS working solution (20 mM EDC, 10 mM s-NHS) by mixing both aliquots and addition 1800 µl of water. Mix thoroughly. Pipet 200 µl/well of the EDC/s-NHS working solution into column 2. Use EDC and s-NHS within 15 minutes of mixing them together!

4.2.8 Leave all remaining wells empty.

4.2.9 Place both the sample plate and hydration plate with biosensors into the Octet®.

4.2.10 Start assay by clicking “GO”.

4.2.11 After completion, transfer Strep-Tactin®XT coated biosensors into an empty biosensor tray. Keep the Strep-Tactin®XT biosensors dry and dark at room temperature until use. Before use, hydrate the Strep-Tactin®XT biosensors for at least 10 minutes in assay running buffer.

Table 1: Octet® program for coating and drying of Strep-Tactin®XT biosensors. ** Column refers to the column in the 96-well plate for the immobilization and drying as shown in figure 1

#	Step	Duration [sec]	Shaker speed [rpm]	Step type	Column**
1	Equilibration	60	1000	Custom	1
2	Activation	300	1000	Activation	2
3	Immobilization	600	1000	Loading	3
4	Quench	300	1000	Quench	4
5	Wash	60	1000	Custom	5
6	Drying	120	1000	Custom	6

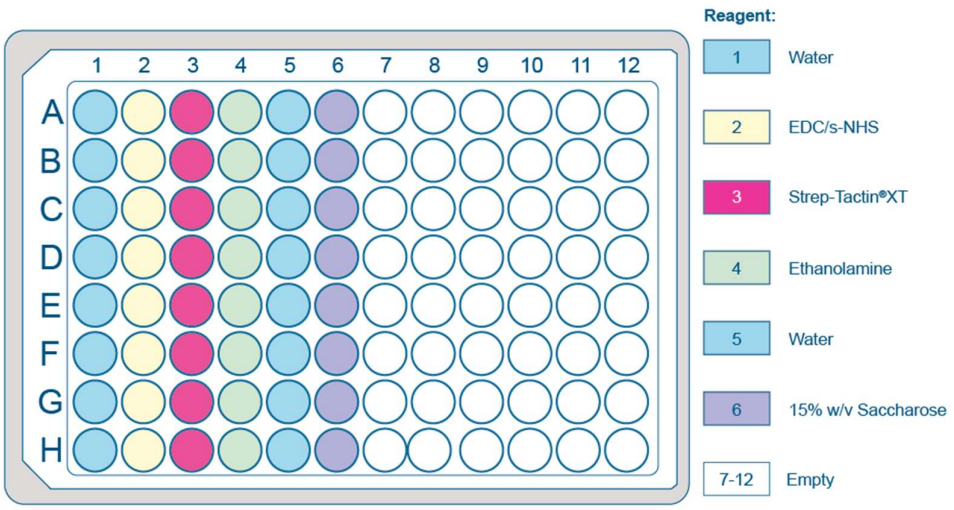

Figure 1: Pipetting scheme of the 96-well for coating of Strep-Tactin®XT biosensors and subsequent drying of the biosensors for later use. The reagent in each column is color-coded and listed in the legend.

4.3 Immobilization of Strep-Tactin®XT to biosensors with subsequent kinetic assay, regeneration and drying.

- Equilibrate reagents and samples (except EDC and s-NHS aliquots) to room temperature prior to preparation. For frozen samples, thaw and mix thoroughly prior to use and chill on ice until needed.
- Use EDC and s-NHS within 15 minutes of mixing them together!
- Ensure that the Octet® instrument is turned on and the lamp is warmed up to room temperature for at least 40 minutes prior to starting the assay.
- Instead of 1x Buffer W other buffers can be applied as assay running buffer. Please note that assay running buffer should be used to dilute the Twin-Strep-tag® ligand and the analyte.

4.3.1 Pre-set the assay as indicated in table 2 in the data acquisition software.

4.3.2 Prepare a 96-well plate for immobilization, assay, regeneration and drying as shown in figure 2.

4.3.3 Pipet 200 µl/well water into column 1 and 11.

4.3.4 Pipet 200 µl/well 1 M ethanolamine pH 8.5 into column 4.

4.3.5 Pipet 200 µl/well 1x Buffer W or your desired assay running buffer into column 5, 7 and 9.

4.3.6 Pipet 200 µl/well Regeneration Buffer into column 10.

4.3.7 Pipet 200 µl/well 15% w/v Saccharose into column 12.

4.3.8 Prepare the Twin-Strep-tag® ligand in assay running buffer and pipet 200 µl/well of the Twin-Strep-tag® ligand into column 6.

4.3.9 Prepare analyte in assay running buffer and pipet 200 µl/well of the analyte into column 8.

4.3.10 Prepare the required volume of Strep-Tactin®XT working solution. Therefore, dilute Strep-Tactin®XT with immobilization buffer to obtain a concentration of 50 µg/ml. Pipet 200 µl/well of Strep-Tactin®XT into column 3.

4.3.11 Prepare 2000 µl of an EDC/s-NHS working solution (20 mM EDC, 10 mM s-NHS) by mixing both aliquots and addition 1800 µl of water. Mix thoroughly. Pipet 200 µl/well of the EDC/s-NHS working solution into column 2. Use EDC and s-NHS within 15 minutes of mixing them together!

4.3.12 Place both the sample plate and hydration plate with biosensors into the Octet®.

4.3.13 Start assay by clicking “GO”.

4.3.14 After completion, transfer Strep-Tactin®XT coated biosensors into an empty biosensor tray. Keep the Strep-Tactin®XT biosensors dry and dark at room temperature until use. Before next use, hydrate the Strep-Tactin®XT biosensors for at least 10 minutes in assay running buffer.

Table 1: Octet® program for coating of Strep-Tactin®XT biosensors and subsequent kinetic assay, regeneration and drying. * Duration of binding of the Twin-Strep-tag® ligand (#6), binding of the analyte (#8) and dissociation of the analyte (#9) depend on the specifications of ligand and analyte and have to be determined experimentally. ** Column refers to the column in the 96-well plate for the immobilization, assay, regeneration and drying as shown in figure 2.

#	Step	Duration [sec]	Shaker speed [rpm]	Step type	Column**
1	Equilibration	60	1000	Custom	1
2	Activation	300	1000	Activation	2
3	Immobilization	600	1000	Loading	3
4	Quench	300	1000	Quench	4
5	Initial baseline	60	1000	Initial baseline	5
6	Binding of Twin-Strep-tag® ligand	custom*	1000	Loading	6
7	Baseline	60	1000	Baseline	7
8	Binding of analyte	custom*	1000	Association	8
9	Dissociation of analyte	custom*	1000	Dissociation	9
10	Regeneration	60	1000	Custom	10
11	Wash	60	1000	Custom	11
12	Conservation	120	1000	Custom	12

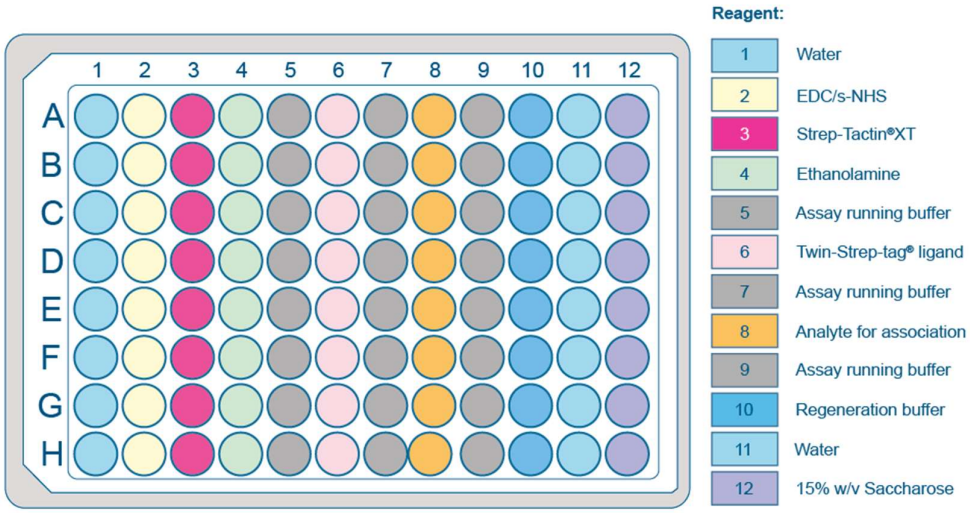

Figure 2: Pipetting scheme of the 96-well plate for coating of Strep-Tactin®XT biosensors and subsequent kinetic assay, regeneration and drying. The reagent in each column is color-coded and listed in the legend.

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US Patent No. 7,981,632
US Patent No. 8,735,540
DE Patent No. 101 13 776
EP Patent No. 1370574

B) STREPTAVIDIN MUTEINS AND METHODS OF USING THEM
US Patent No. 10,065,996
US Patent No. 11,168,116
EP Patent No. 2 920 204
CN Patent No. 3481362
AU Patent No. 2017257203
JP Patent No. 6475630

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