Gator® Strep-Tactin XT

PRODUCT INSERT
Part Number: 160033

Overview

Gator® Strep-Tactin XT probes are designed to capture proteins tagged with Twin-Strep-tag® (TST). They are coated with Strep-Tactin® XT, that binds to Twin-Strep-tag (TST) with pM affinity. Strep-Tactin® XT has been engineered from Streptavidin by IBA-Lifesciences GmbH and is also used in protein purification columns to purify TST fusion proteins.

TST is commonly used for the purification of recombinant proteins in various applications, such as structural biology, enzyme engineering, and protein characterization. Due to the highly specific binding of Twin-Strep-tag® it provides an efficient and reliable method for obtaining highly pure proteins with minimal contamination. It is better than His-tag for proteins that need to be purified at physiological pH to retain functionality.

The Strep-Tactin XT probe is useful for quantitation and kinetic characterization of proteins tagged with TST.

Product Information

Materials Required

- Gator Max Plate, PN: 130062
- Gator BLI 96-Flat Plate, PN: 130260
- Precision Pipettes, User Supplied
- Sterile Pipette Tips, User Supplied

Storage Conditions

Store the tray in its foil packaging pouch at room temperature (RT), ensuring that the zipper is fully sealed. Probes are stable at RT for 1 year.

General Methods

Sample Volume

Max Plate: 250 - 280 µL/well
96-well Black Plate: 180 µL - 200 µL/well
384-well Black Plate: 80 µL - 100 µL/well

Prewet Conditions

250 µL/well in Max Plate for 10 minutes at 1000 rpm in buffer diluent

Kinetic Analysis of TST Fusion IL-17A & IL-17F Heterodimer Proteins

Loading of TST fusion Human IL-17A & IL-17F heterodimer protein and subsequent association and dissociation of different concentrations of human IL-17 RA.

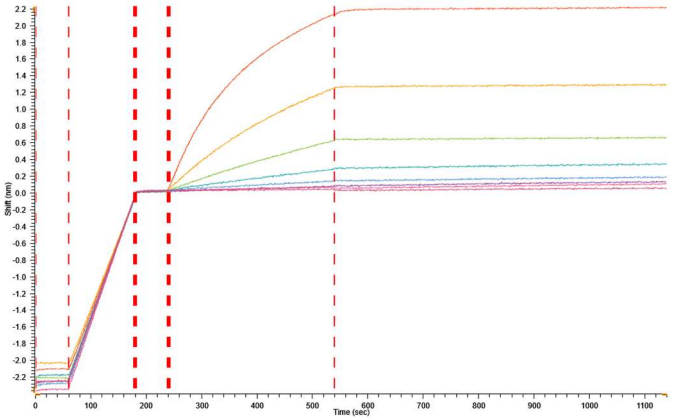

Fig. 1. The graph shows a kinetic experiment between TST fusion human IL-17A & IL-17F heterodimer protein and human IL-17 RA. Following a baseline measurement in K Buffer, TST fusion IL-17A & F (1 µg/mL in K Buffer) was loaded onto Strep-Tactin XT probes (1000 rpm; 120 sec) followed by association and dissociation of IL-17 RA at 500, 167, 56, 19, 6, 2, 0.7, and 0 nM. K_D = 1.99 nM.

Strep-Tactin XT Probe Shows Comparable Performance in Crude and Purified Samples

Strep-Tactin XT probe can be used for quantitation of TST fusion proteins in purified buffers and in cell lysates and cell culture media. The probes can quantify proteins down to low µg/mL and can be regenerated up to 10 times with no loss in binding capacity using Gator® Regen Buffer (PN: 120063).

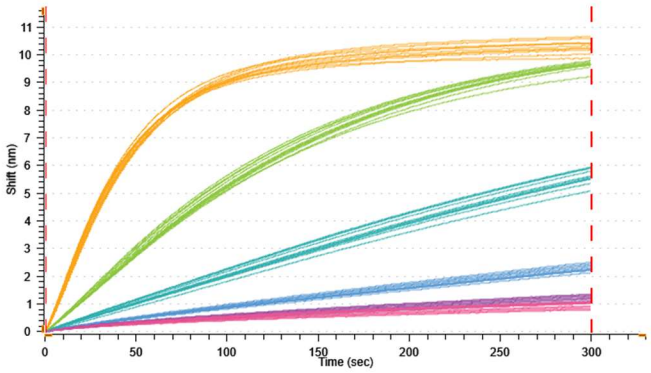

Fig. 2. Graph shows quantitation of TST fusion GFP P (27 kDa). TST GFP was spiked into HEK293 cell lysate (Expi293TM media; spun down; diluted 1:100 in Q buffer). Each concentration was measured at 10 replicates from regenerated biosensors using Gator Regen Buffer.

Conc. (ug/mL)	Avg. Binding Rate	% CV (n=10)	Avg. Calculated Conc. (ug/mL)	% CV (n=10)
6.67	0.232	3.6	6.350	3.4
2.22	0.072	4.8	2.219	4.2
0.74	0.019	6	0.689	5.1
0.25	0.005	4.2	0.227	3.5
0.08	0.001	4.9	0.071	5.6
0.03	0.000	13.1	0.023	15.9

Table 1: Accuracy and precision of quantifying TST GFP using the Strep-Tactin XT probe. Concentrations were calculated using initial binding rate

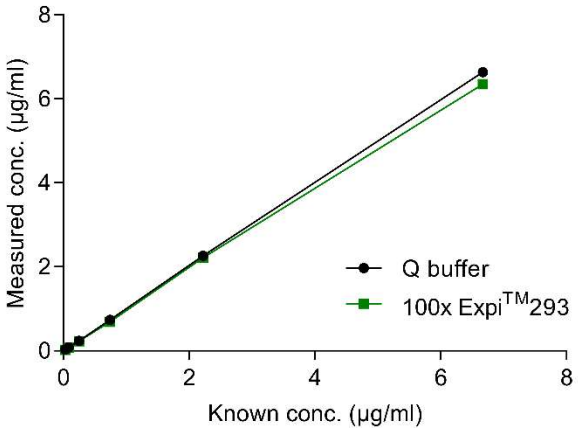

Fig. 3. Standard curve for TST GFP in Q buffer vs in cell lysate from cells grown in Expi293 media diluted 1:100 in Q buffer.

Strep-Tactin XT Probe has Minimal Interference from Biotin

The Strep-Tactin XT probe can tolerate up to 0.1 mM of biotin, which is an important component of most cell culture media. This is 10 times higher than the biotin content in most mammalian cell culture media (Ref 1). In case of interference from biotin or biotinylated proteins,1 mg/mL avidin blocking solution can be added to the sample to quench the interference.

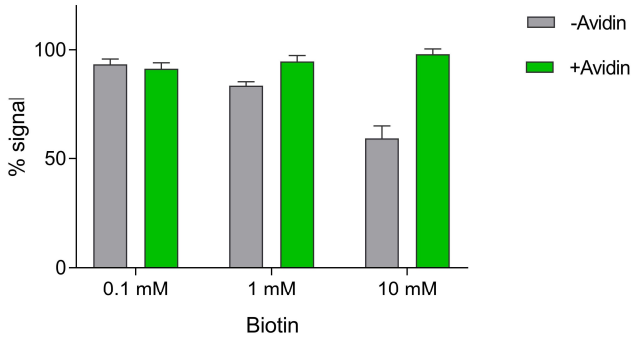

Fig. 4. % Signal in presence of 0.1, 1, and 10 mM biotin. 1 mg/mL avidin can be used to prevent the interference from biotin

Ref 1. Protocol: Blocking of biotin in cell lysate and culture media. IBA-Lifesciences gmbh.

