

# Outstanding performance from purification to immobilization – Strep-tag® system

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The Strep-tag® system is a versatile protein purification, detection and immobilization platform. It is well known for its outstanding performance providing exceptionally pure proteins. The Strep-Tactin®XT provides a remarkable binding affinity in low pM ranges while maintaining its binding reversibility and mild recovery of immobilized proteins. This allows protein purification at high yields and purity, even with challenging proteins as well as from mammalian expression systems (e. g. Expi). Furthermore, it fulfills the high demands of downstream applications such as SPR, covering all steps from purification to immobilization efficiently.

## AFFINITY – A COMPROMISE BETWEEN PURIFICATION AND ASSAY

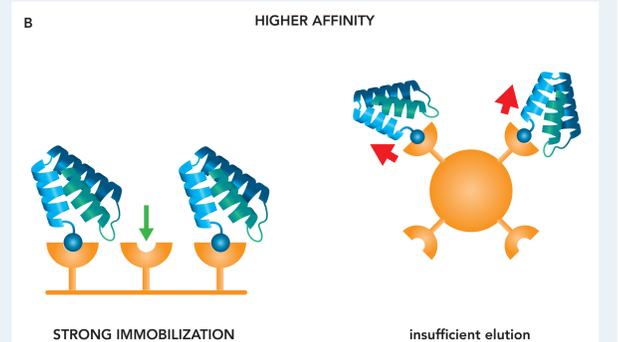
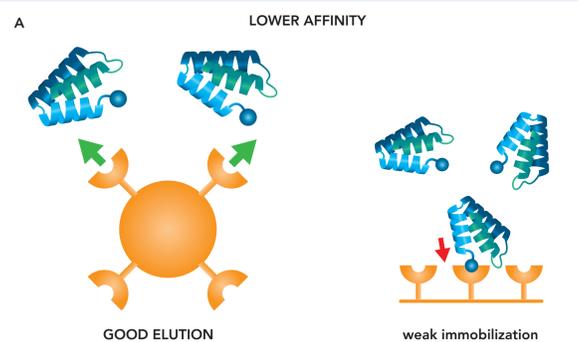
The affinity of a tag to its ligand is an important property depending on the application.

### (A) Protein purification:

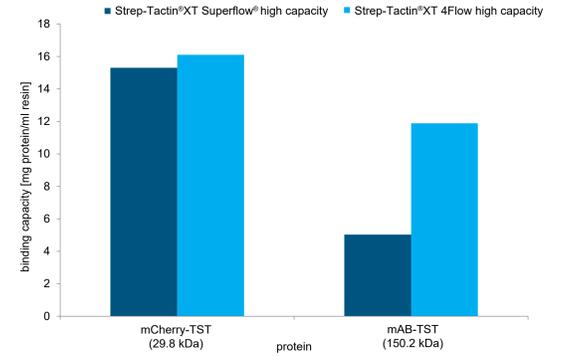
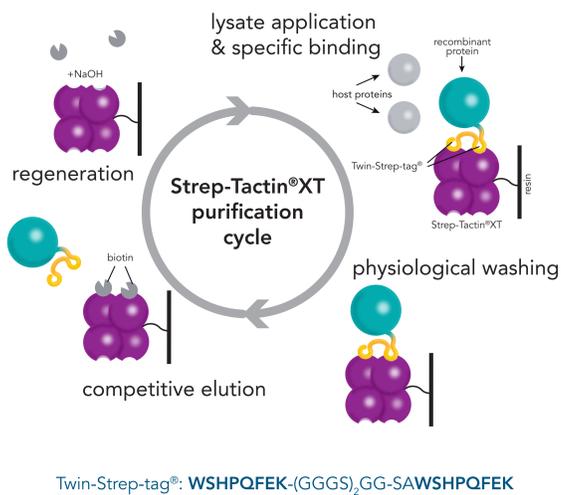
The affinity of the tag should allow an efficient elution from its ligand.

### (B) Analytic applications (immobilization):

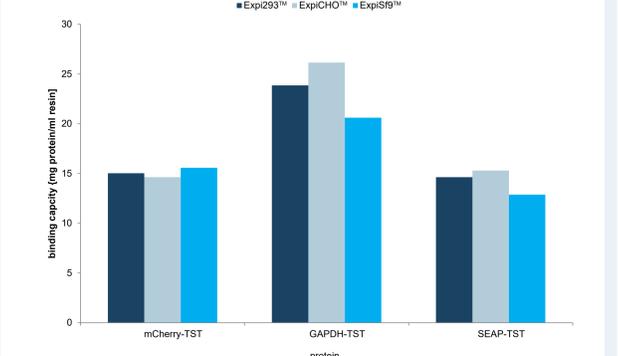
The tag must have an affinity that is high enough to bind efficiently to the ligand - even under challenging conditions.



Purification

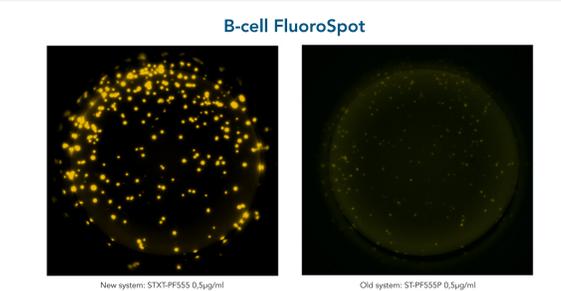


**Strep-Tactin®XT 4Flow – Higher capacity for large proteins.** Twin-Strep-tag® proteins of different size (mCherry-TST, 29.8 kDa and mAB-TST, 150.2 kDa) were spiked in buffer W and purified with either Strep-Tactin®XT Superflow® high capacity or Strep-Tactin®XT 4Flow high capacity. Purification with Strep-Tactin®XT 4Flow high capacity provides higher yields for the large mAB protein.

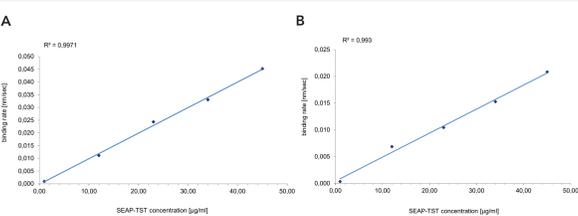


**Excellent binding capacity of Twin-Strep-tagged proteins.** Three different TST-proteins were purified via Strep-Tactin®XT 4Flow high capacity from different Expi supernatants (Expi293™, ExpiCHO™ and ExpiSf9™). The max. binding capacity was determined for each protein by eluting overloaded gravity flow columns.

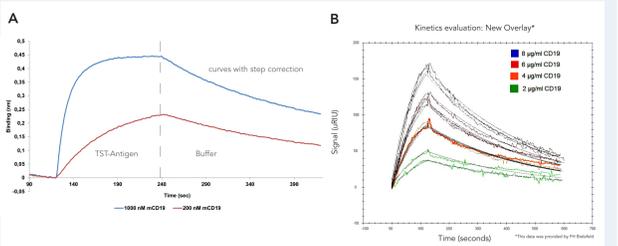
Immobilization



**Comparing signal strength when using fluorescently labeled Strep-Tactin® or Strep-Tactin®XT for detection in reversed B-cell FluoroSpot assay.** Immobilized anti-human IgG antibodies are used to capture IgG secreted by B cells. IgG secreted by B cells is then allowed to bind antigens labeled with Twin-Strep-tag®. By finally using fluorescently labeled Strep-Tactin® or Strep-Tactin®XT the position of antigen-specific B cells can be detected as a spot on the membrane.



**Measurement of protein concentration in Expi supernatants using Strep-Tactin®XT Dip and Read Biosensors on a BLItz® device.** ExpiCHO™ (A) and Expi293™ (B) supernatants were spiked with five different SEAP-TST concentrations. Calibration curves show a high coefficient of determination ( $R^2$ ) for a linear fit. Therefore, Strep-Tactin®XT Biosensors can be used for reliable high throughput determination of protein concentration in high density cell culture media like Expi.



Method	$K_D$ (M)	$k_{on}$ (1/Ms)	$k_{off}$ (1/s)	$\chi^2$	$R^2$
BLItz®	$6.234 \times 10^{-8}$	$6.614 \times 10^4$	$4.123 \times 10^{-3}$	0.0266	0.9993
SPR	$6.27 \times 10^{-8}$	$4.55 \times 10^4$	$2.85 \times 10^{-3}$	14.82	

**Binding kinetic of an anti-mouse CD19 nanobody to mouse CD19 receptor.** Kinetic was determined with BLItz® (A) and SPR (B) using Strep-Tactin®XT coated chips or biosensors. The nanobody and the antigen contained a Twin-Strep-tag®.

