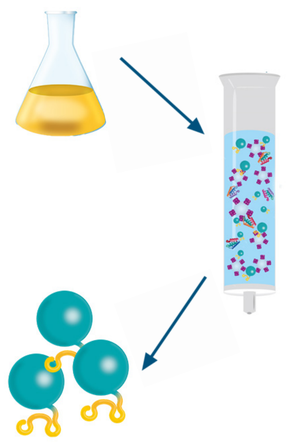
The Twin-Strep-tag® - A universal protein tag for antibody discovery & development

Philipp Henning, Thomas Pohl, Christos Gouloudis, Alexandra Nordlohne, Fabian Mohr[1]

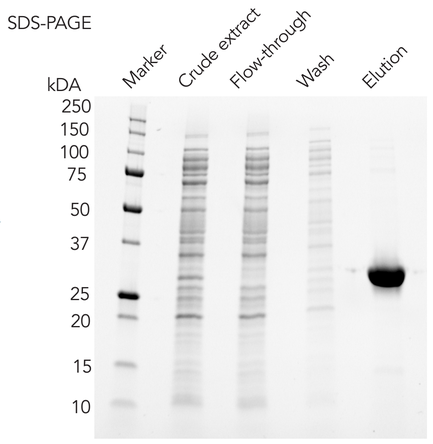
[1] IBA Lifesciences GmbH, Rudolf-Wissell-Str. 28, 37079 Goettingen, Germany

Due to their ability of specific and high affinity target binding, antibodies are used for disease treatment and as research tool. The identification of well-working antibodies is a complex procedure in which the target antigen plays a central role. Fusing the antigen to a short peptide tag, the Twin-Strep-tag®, enables its use for different development steps, including analytics and screening applications. Thereby the whole procedure becomes more time- and cost-effective.

Antigen expression & purification: Manual or automated purification



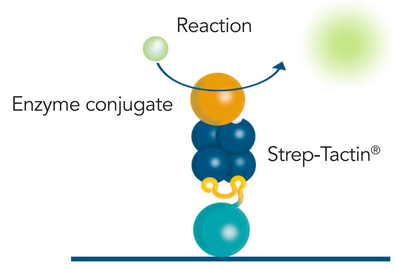
The antigen of interest has to be expressed with a Twin-Strep-tag® (TST) in a suitable host (e.g. in *E.coli* cells) and purified. Automated high-throughput purifications using magnetic beads are possible.



Expression Scale:
E.coli 10 L fermentation
Yield:
0.3 g/L of purified protein

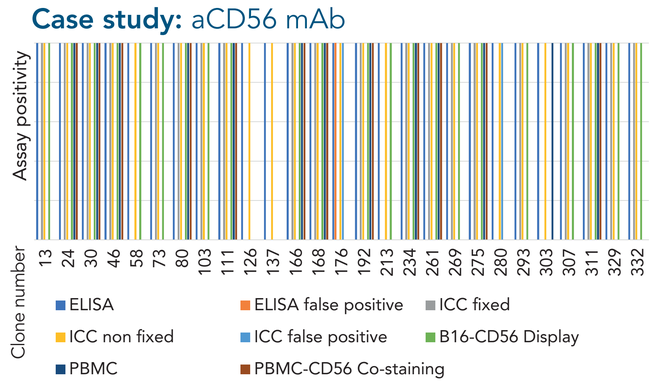
The expression and purification of a 27 kDa protein resulted in highly pure (94%) protein with a sufficient yield for immunization.

Antigen analysis: ELISA, Western Blot, FACS

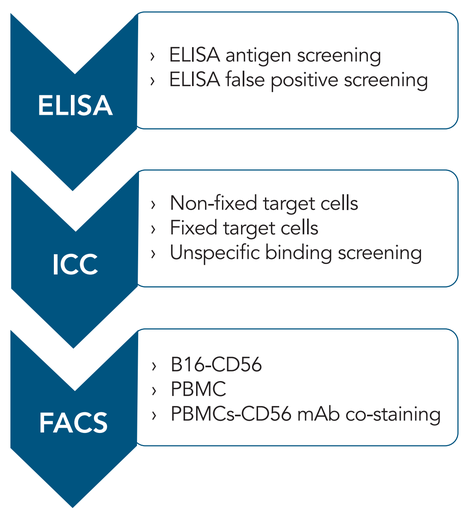


Several different detection reagents that bind to the TST allow for analysis of the expressed antigen via e.g. ELISA or Western blot.

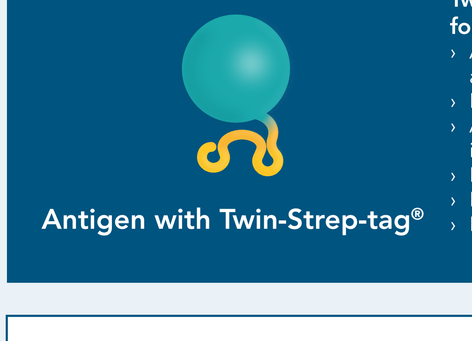
Case study: aCD56 mAb



Results of the screening process for selected clones. The result is either positive or negative.



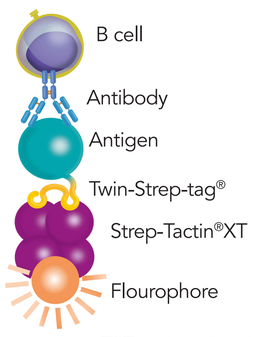
Workflow for mAb screening. During ELISA specificity and unspecific binding of the mAb is established. Therefore TST-labeled target protein and TST-labeled-control proteins are bound to a Strep-Tactin®XT coated 96 well plate. ICC is performed on fixed cells and non-fixed cells to determine the compability of the target mAbs with fixation. Additionally target unspecific binding is tested. Finally FACS screening on primary target cells (PBMCs) and artificial target-over expressing cells (B16) is performed.



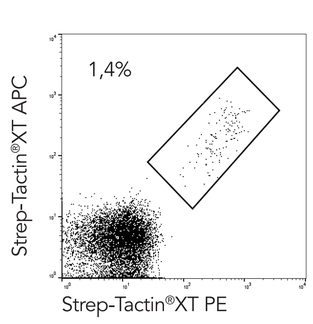
Antigen with Twin-Strep-tag®

Twin-Strep-tag® - a universal tag for:
- Antigen (protein) purification and analysis
- Immunization
- Antigen-specific B cell staining and isolation
- ELISA and Western blot
- Biosensor applications
- Biopanning

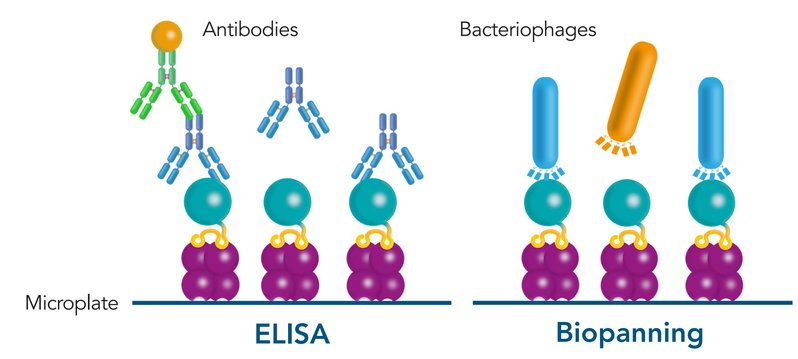
B cell staining & sorting: FACS



The previously expressed TST-antigen is suitable for antigen-specific B cell staining. For this application, the antigen hast to be combined with a fluorescent Strep-Tactin®XT conjugate of choice.



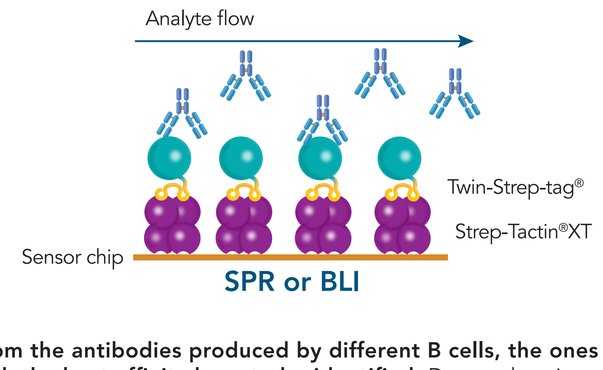
Double staining of a specific cell population using a protein fused to a Twin-Strep-tag® in combination with Strep-Tactin®XT PE and APC.



Possible screening applications include ELISA or biopanning. Antigens can be immobilized via their Twin-Strep-tag® on Strep-Tactin®XT coated microplates. Subsequently, antibodies or antibody fragments can be screened for their antigen-binding.

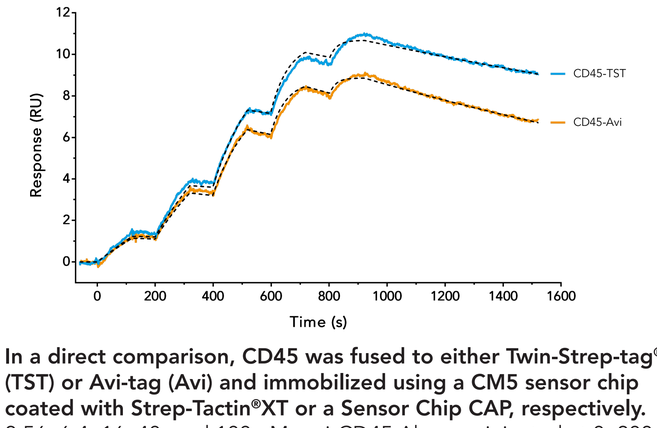
The picomolar affinity of Strep-Tactin®XT to the Twin-Strep-tag® permits the use for biosensor assays such as SPR to evaluate the affinity to a target.

Screening applications



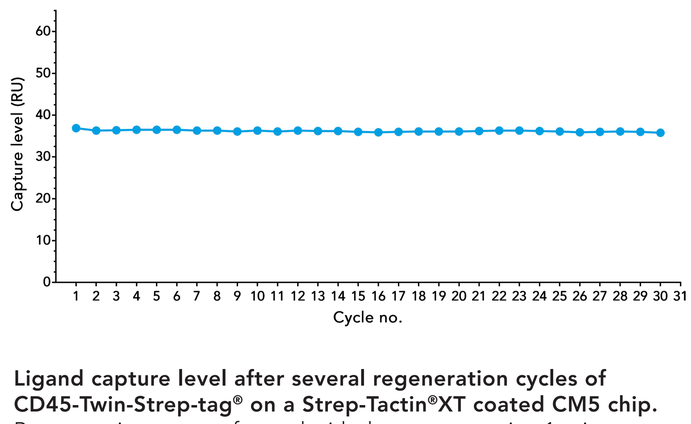
From the antibodies produced by different B cells, the ones with the best affinity have to be identified. Due to the picomolar affinity of the Twin-Strep-tag® to Strep-Tactin®XT, this interaction is suitable for using it for kinetic measurements similar to the Avi-tag – streptavidin interaction.

Comparison of Anti-CD45 Antibody interaction with Twin-Strep-tagged and Avi-tagged CD45



In a direct comparison, CD45 was fused to either Twin-Strep-tag® (TST) or Avi-tag (Avi) and immobilized using a CM5 sensor chip coated with Strep-Tactin®XT or a Sensor Chip CAP, respectively. 2.56, 6.4, 16, 40, and 100 nM anti-CD45 Ab were injected at 0, 200, 400, 600, and 800 s. Both systems delivered similar results.

Ligand capture level



Ligand capture level after several regeneration cycles of CD45-Twin-Strep-tag® on a Strep-Tactin®XT coated CM5 chip. Regeneration was performed with three consecutive 1-minute injections of 3 M GuHCl.

SUMMARY

The Strep-tag® system is well known for protein purification, but provides many tools beyond this application. Due to its highly specific and high affinity tag-ligand interaction, it offers a versatile platform for antibody development. The need for only one type of tag for key processes such as antibody discovery, screening and production simplifies the whole procedure, making it more time- and cost-effective.

www.iba-lifesciences.com