How a single protein tag provides a platform for key processes in antibody production

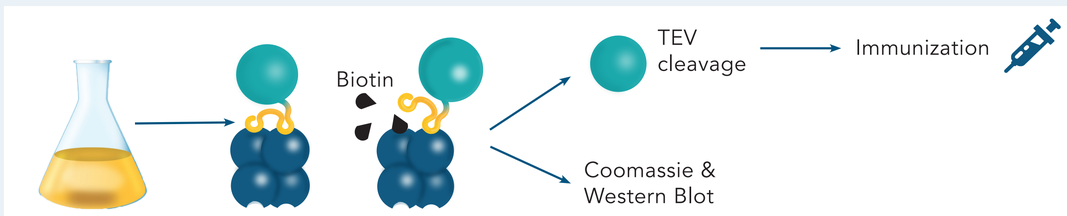
Dr. Fabian Mohr[1], Fabian Risse[2]

[1]IBA Lifesciences GmbH, Rudolf-Wissell-Str. 28, 37079 Goettingen, Germany
[2] XanTec bioanalytics GmbH, Merowingerplatz 1a, 40225 Duesseldorf, Germany

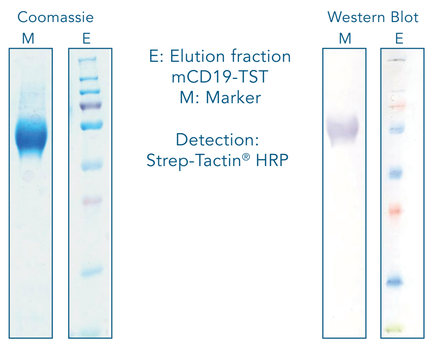
Antibodies play an important role in immune responses and serve as treatment options for infections or diseases. The identification of well-working antibodies is a complex procedure that involves several different steps. These include the initial production of the antigen, immunization of a host, retrieving the produced antibodies as well as screening for promising candidates.

The Strep-tag® technology provides options to centralize various steps of antibody development onto one platform. Different tag-ligand combinations with micromolar to picomolar affinities enable the use of this system for a wide range of required assays that are needed for antibody validation. Thereby it helps to save time and reduce the costs of the whole procedure.

ANTIGEN PRODUCTION & ANALYSIS

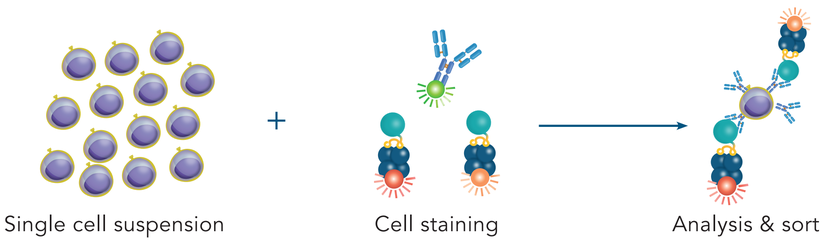


The antigen of interest has to be expressed with a Twin-Strep-tag® (TST) in a suitable host (e.g. in HEK or CHO cells) and purified. Optionally, the TST can be removed by TEV cleavage for further assays and immunization.

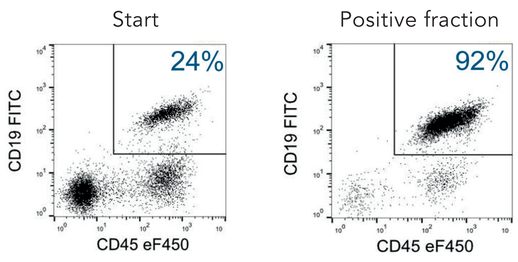


The expression and purification of a Twin-Strep-tagged murine CD19 antigen via Strep-Tactin® resulted in 3.2-7.2mg/l of the highly pure antigen for immunization.

ANTIGEN-SPECIFIC B CELL ISOLATION



Cell staining should include antibodies against unwanted populations and double-staining for antigen-specific B cells to minimize false positives. Optionally, B cells can be pre-enriched to relatively increase the proportion of antigen-specific cells.

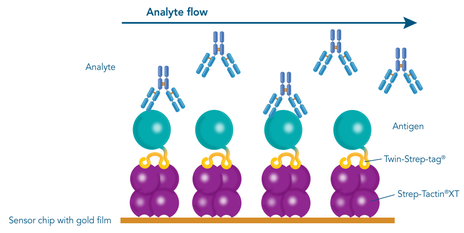


Optional pre-enrichment: The FACS plots show the percentage of CD19⁺ B cells before and after isolation from murine splenocytes. The resulting pure cell population is suitable for further downstream selection procedures.

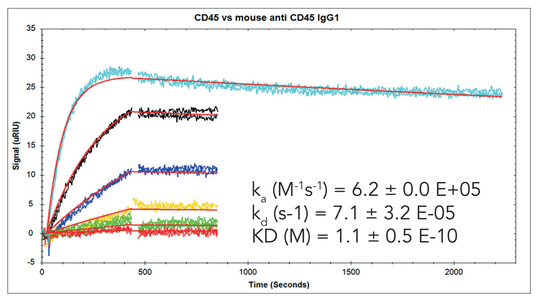
To isolate the B cells that produce the antibodies of interest, the same antigen that was purified for the initial immunization can be used. It binds to fluorescent Strep-Tactin® conjugates via the Twin-Strep-tag®. This way, a staining reagent is formed that is suitable for sorting antigen-specific B cells via flow cytometry.

VALIDATION OF TARGET ANTIBODIES

› Surface Plasmon Resonance (SPR)



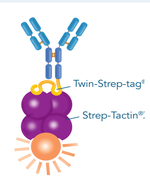
The picomolar affinity of Strep-Tactin®XT to the Twin-Strep-tag® permits the use for biosensor assays such as SPR to evaluate the affinity to a target.



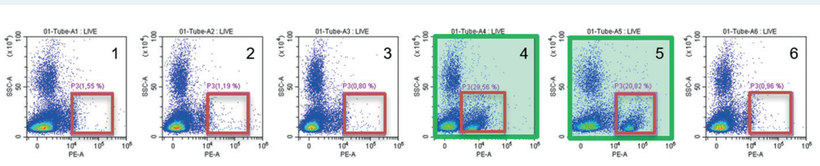
From the antibodies produced by different B cells, the ones with the best affinity have to be identified. The graph shows the affinity of an CD45 antibody against hCD45, which was immobilized via a Twin-Strep-tag®. Different ligand concentrations were tested with n =2 measurements each.

(Data provided by XanTec bioanalytics GmbH)

› Flow cytometry staining



As an additional functionality test for the selected antibodies, flow cytometry for efficient cell staining can be performed. For this approach the selected antibodies have to be sequenced and can be fused to a Twin-Strep-tag®.



The FACS plots show that two out of six tested clones efficiently stained cells, indicating a good accessibility of the epitope and identifying these antibodies as promising candidates for further evaluation.

SUMMARY

The Strep-tag® platform is well suited in the process of antibody development:

› It provides highly pure antigens for immunization in a simple one-step procedure.
› The exceptional high specificity and affinity are ideally suited for antigen and antibody analysis.

www.iba-lifesciences.com