Protein Production & Assays

For research use only

Protocol

# ELISA with Strep-Tactin® or StrepMAB conjugates

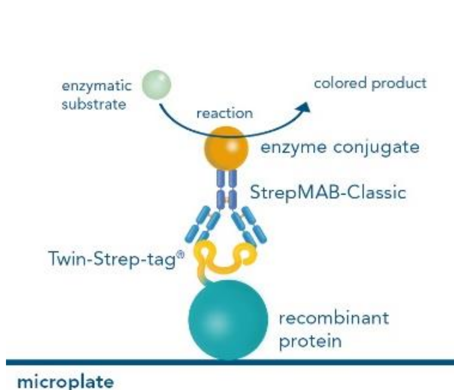
Detection of Strep-tag®II and Twin-Strep-tag® fusion proteins in microplate based assays

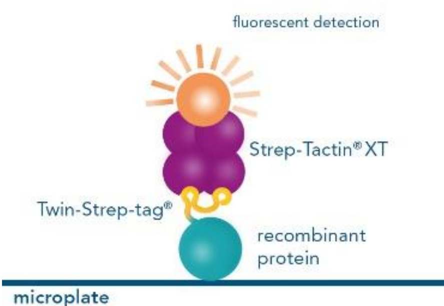
## 1 DESCRIPTION

The enzyme-linked immunosorbent assay (ELISA) is a commonly used method for detection and quantification of ligands, such as proteins, peptides, or hormones. In an ELISA, the target ligand or capturing reagent is immobilized on a surface. The essential components include a ligand of interest, an antibody or detection reagent for specific recognition of the ligand, and an enzymatic reporter for converting a substrate into a measurable signal.

IBA Lifesciences provides various ELISA products suitable for direct and sandwich assays, each offering different experimental conditions as illustrated in Figure 1. While this protocol offers a set of experimental conditions and guidelines for these different ELISA approaches, it is essential to adjust the conditions to your specific Strep-tag®II or Twin-Strep-tag® fusion protein and other experimental requirements.

**Direct ELISA**




**Sandwich ELISA**

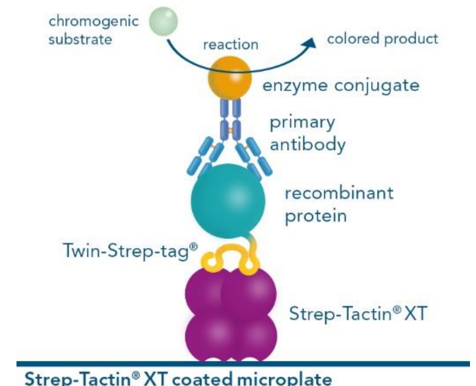



*Figure 1: Different detection approaches in ELISA*

# 2 GENERAL INFORMATION AND RECOMMENDED MATERIAL

## 2.1 Detection limits, reporter conjugate dilution and signal strength

Detection limits and signal strength are dependent on the specific protein and experimental setup. In general, larger proteins (>100 kDa) can be more easily detected than smaller proteins (up to 50 kDa). Figure 2 shows a comparison of two Twin-Strep-tag® fusion proteins as detected by a 1:5000 dilution of Strep-Tactin®-HRP. Trends shown here could be reproduced with all IBA Lifesciences reporter conjugates.

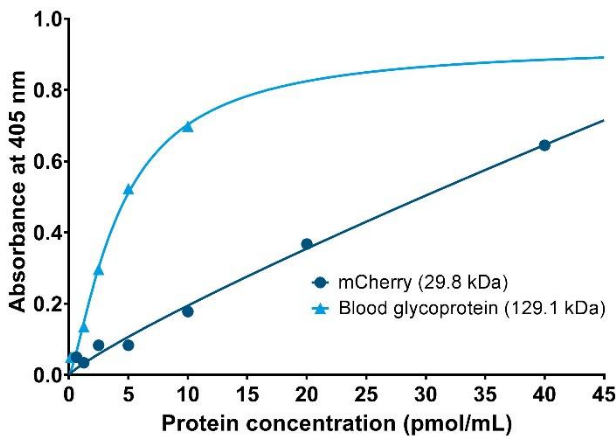



*Figure 2: Detection limit of different sized proteins, measured with Strep-Tactin® HRP*

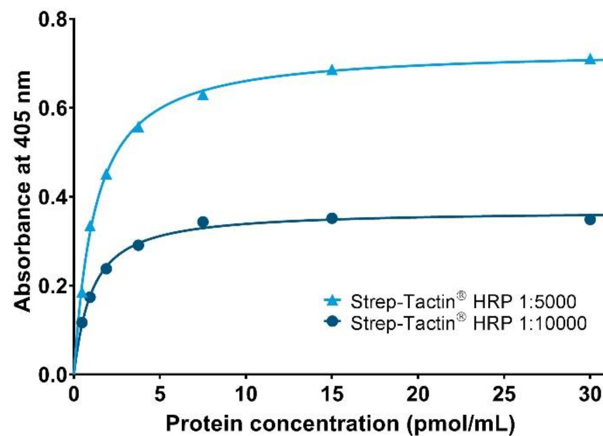



*Figure 3: Signal strength at different reporter conjugate dilutions, measured with a 150 kDa protein*

In general, a factor 2 dilution of reporter conjugate does not largely influence the detection limit, but rather affects the maximum signal and signal plateauing. A representative example is shown in Figure 3, where a Twin-Strep-tag® fused rat IgG antibody was detected in different concentrations by Strep-Tactin® HRP in a 1:5000 and a 1:10000 dilution. For fluorescent dye conjugates, the signal does not change significantly at low protein amounts. This trend could be reproduced with all our tested proteins.

## 2.2 Biotin in your sample

Do not use skimmed milk for blocking when detecting with Strep-Tactin® or Strep-Tactin®XT conjugates. Skimmed milk contains biotin, which binds to Strep-Tactin® and Strep-Tactin®XT and will result in high unspecific signal. Instead of skimmed milk, please use bovine serum albumin (BSA) as listed in section 2.3 or another biotin-free buffer of your choice as a blocking reagent.

Biotinylated proteins will stain sensitively when Strep-Tactin® or Strep-Tactin®XT conjugates are used for detection. To prevent this, make sure to block the biotin by adding avidin to your sample. StrepMAB-Classic HRP does not bind biotinylated proteins or biotin and therefore application of avidin is not required.

### 2.3 Required materials

The materials listed below were used to establish the protocol under section 3 and to obtain detection limits given in our product datasheets.

#### 2.3.1 Recommended products from other suppliers

| Product name | Application | Supplier |
|---|---|---|
| Strip plate, 12xF8, PS, F-bottom clear, microlon, med. binding | Enzymatic detection in direct ELISA assays | Greiner Bio-One |
| Microplate, 96 well, PS, F-bottom (chimney well) µClear®, black, high binding, sterile | Detection with fluorescent dye conjugates | Greiner Bio-One |
| 1-Step™ PNPP Substrate Solution | Chromogenic detection with StrepTactin®-AP | Thermo Fisher Scientific |
| 1-Step™ ABTS Substrate Solution | Chromogenic detection with HRP-conjugates | Thermo Fisher Scientific |

#### 2.3.2 Recommended Buffers for ELISA

| Buffer/Solution | Composition |
|---|---|
| Coating buffer | 50 mM $NaHCO_3$<br>pH 10.5 |
| TBS buffer | 50 mM Tris/HCl, pH 7.4<br>140 mM NaCl |
| TBS-Tween buffer | TBS buffer<br>0.1% w/v Tween 20 |
| TBS-blocking buffer | TBS-Tween buffer<br>2% BSA<br>0.05% w/v Tween 20 |

#### 2.3.3 Products by IBA Lifesciences

| Reporter conjugate | Cat. No. | Dilution range | Recommended starting dilution |
|---|---|---|---|
| **Enzyme conjugates** | | | |
| Strep-Tactin®-AP | 2-1503-001 | 1:500-1:5000 | 1:1600 |
| Strep-Tactin®-HRP | 2-1502-001 | 1:2000-1:10000 | 1:5000 |
| StrepMAB-Classic HRP | 2-1509-001 | 1:2000-1:5000 | 1:2500 |
| **Fluorescence conjugates** | | | |
| StrepMAB-Classic fluorescent dye conjugates can also be used in ELISA, but we recommend using Strep-Tactin®XT or StrepMAB-Immo conjugates due to a better performance. | | | |
| Strep-Tactin®XT | | | |
| DY-488 | 2-1562-050 | 1:500-1:2000 | 1:1000 |
| DY-549 | 2-1565-050 | 1:500-1:2000 | 1:500 |
| DY-649 | 2-1568-050 | 1:500-1:2000 | 1:500 |
| StrepMAB-Immo | | | |
| DY-488 | 2-1564-050 | 1:500-1:2000 | 1:500 |
| DY-549 | 2-1567-050 | 1:500-1:2000 | 1:1000 |
| DY-649 | 2-1570-050 | 1:500-1:2000 | 1:1000 |

# 3 PROTOCOL

## 3.1 Chromogenic and fluorescence detection in direct ELISA

Please note the differences in handling for the various conjugates.

**3.1.1** To immobilize the target protein on the well plate, dilute the protein in coating buffer to your desired concentration. Add 100 µL of the diluted protein sample per well. Cover and incubate for 1 h at room temperature or at 4 °C over night.

- For assays with fluorescent dye conjugates, use a black plate with a clear bottom.

**3.1.2** Invert the plate and tap on absorbent paper to remove excess liquid. Add 300 µL of TBS-blocking buffer per well, cover and incubate for 2 h at room temperature.

**3.1.3** Wash 2x with 300 µL TBS-Tween buffer and 1x with 300 µL TBS buffer, remove excess liquid by inverting the plate.

**3.1.4** To detect the Strep-tag® fusion proteins, dilute the reporter conjugates as follows:

- For Strep-Tactin® enzyme conjugates, Strep-Tactin®XT or StrepMAB-Immo fluorescent dye conjugates, dilute them in TBS buffer.
- For StrepMAB-Classic HRP conjugate, dilute it in blocking buffer.

Start with the dilutions listed in the table in section 2.3.3. Add 100 µL of the diluted reporter conjugate per well.

Cover and incubate for 1 h at room temperature.

- For fluorescent dye conjugates, use an opaque cover foil.

**3.1.5** Wash 2x with 300 µL TBS-Tween buffer.

- For AP and HRP conjugates, wash 2 more times with 300µL TBS buffer.
- For fluorescence conjugates, wash only 1 more time with TBS buffer, then add 100 µL TBS buffer.

**3.1.6** To measure the protein concentration, develop the plate according to your needs, as described by the substrate manufacturer.

- Excitation and emission maxima for fluorescence conjugates can be found in the respective data sheets.

## 3.2 Sandwich ELISA with Strep-Tactin® and Strep-Tactin®XT coated microplates

**3.2.1** To bind your target protein to the Strep-Tactin® ligand, add 150 µl/well of your protein sample in TBS buffer, in the desired concentration. Incubate for 1-2 h at room temperature, then invert the plate and tap on absorbent paper to remove excess liquid.

**3.2.2** Block by adding 300 µL/well of blocking buffer. Incubate at room temperature for at least 30 minutes.

**3.2.3** Wash 3x with 300 µL/well TBS-Tween buffer, and 1x with 300 µL/well TBS buffer.

**3.2.4** Add your desired detection antibody in 150 µL/well and incubate 1 hour at room temperature.

**3.2.5** Wash 2x with 300 µL TBS-Tween buffer and 2x with 300 µL TBS buffer.

**3.2.6** To measure the target protein concentration, develop the plate as described in the manufacturer's instructions for your chosen reporter conjugate.

# 4 TROUBLESHOOTING

## 4.1 No signal

| | |
|---|---|
| Not enough reporter conjugate is bound to the protein of interest. | Use more concentrated reporter conjugate. Incubate longer (e.g., overnight) at 4 °C. |
| Insufficient antigen. | Try the recommended starting concentration for proteins of a similar size as your antigen. The detection limits of different sized proteins can be found in the conjugates' corresponding data sheets. |
| The protein of interest is not abundantly present in the tissue. | Use an enrichment step to maximize the signal (e.g., prepare nuclear lysates for a nuclear protein, etc.). |
| Excessive washing of the plate | Do not let a single washing step incubate for more than 5 minutes. Develop the plate as soon as possible after the last washing step. |
| Too much blocking does not allow you to visualize your protein of interest. | Switch blocking reagents or block for less time, we recommend 3% BSA and 0.05% v/v Tween 20 in TBS for 2 h. |
| StrepMAB-Classic HRP or Strep-Tactin® HRP inhibited by sodium azide. | Do not use sodium azide together with HRP conjugates. |
| Detection solution is old, and substrate is inactive. | Use fresh substrate. |

## 4.2 Unspecific signal

| | |
|---|---|
| Blocking of non-specific binding might be absent or insufficient. | Increase the blocking incubation period and consider changing blocking agent. We recommend 3% BSA and 0.05% v/v Tween 20 in TBS for at least 1 h. |
| Concentration of reporter conjugate may be too high. | Titrate the reporter conjugate to the optimal concentration, incubate for longer but in more dilute reporter conjugate. |
| Washing of unbound reporter conjugate may be insufficient. | Increase the number of washes after reporter conjugate incubation. |
| Your choice of microplate may give high background under your conditions. | Try a microplate with lower binding affinity. |

## 4.3 Highly divergent duplicate measurements

| | |
|---|---|
| Scattering and fluctuating signal due to any cause | Enzymatic detection: Measure activity (1 measurement per minute immediately after substrate addition) instead of a single measurement after incubation, to identify wells with highly fluctuating signal and exclude these.<br>Fluorescence detection: Measure multiple times consecutively and exclude wells that show strong fluctuation |
| Contamination during emptying of plate, often optically visible | Tap the plate on a clean piece of absorbent paper after every pipetting step, especially during washing after incubation with reporting conjugate. |
| Presence of detergent causing scattering of signal | Add extra washing step(s) with TBS buffer to remove all the detergent |
| Air bubbles in the sample causing scattering of signal | Avoid air bubbles, for example by using the inverse pipetting technique |

### 4.4 Concentration series of (Twin-)Strep-tag® fused proteins shows no differences between dilutions

| | |
|---|---|
| If signal is relatively high (>OD 0,5 for chromogenic enzymatic reactions): Your protein concentration series only encompasses values that make no difference in signal | Dilute your protein further and run dilution series until you see differences in signal between different protein concentrations. For enzymatic reactions, let the reaction run longer to let differences between protein amounts develop. |
| If signal is relatively low (~OD 0,1): your protein does not exceed the amount of unspecific binding of the reporter conjugate | Increase amount of protein, and/or see remedies under 4.2. |

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for the latest version of this manual.

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**www.iba-lifesciences.com/patents-licenses-trademarks.html**

If you have any questions, please contact

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We are here to help!

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