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Protocol

# Western Blot with Strep-Tactin® or StrepMAB conjugates

For detection of Strep-tag®II and Twin-Strep-tag® fusion proteins

## 1 DESCRIPTION

Western blotting is a convenient method to detect Strep-tag®II and Twin-Strep-tag® proteins after protein purification or in complex samples. The sample containing the Strep-tagged protein is at first separated via SDS-PAGE and then transferred to an appropriate membrane. Detection of the target protein can occur with a Strep-tag® specific antibody, StrepMAB-Classic, or Strep-Tactin®. Both StrepMAB-Classic and Strep-Tactin® are provided as conjugates with enzymes, horse horseradish peroxidase (HRP) or alkaline phosphatase (AP). However, if a a specific detection reaction or secondary antibody should be used StrepMAB-Classic is also available non-conjugated. The following protocol describes chemiluminescent or chromogenic detection with Strep-Tactin® HRP and StrepMAB-Classic and chromogenic detection with Strep-Tactin® AP.

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# 2 GENERAL INFORMATION AND REQUIRED MATERIAL

For optimal results, we recommend the use of nitrocellulose membrane. When detecting with Strep-Tactin®, do not use skimmed milk for blocking since it contains biotin which would bind to Strep-Tactin® and therefore produce a high background. Instead of skimmed milk, please use BSA as blocking reagent.

The blocking of biotinylated proteins (e.g., the biotin carboxyl carrier protein (BCCP, 22 kDa) in case of *E. coli*) is optional. They will stain sensitively, when Strep-Tactin® conjugates are used for detection. Biotin Blocking Buffer can be easily prepared using by dissolving 2,5 mg/ml Avidin (cat. no. 2-0204-015) in PBS buffer. However, StrepMAB-Classic do not bind biotinylated proteins or biotin and therefore application of Biotin Blocking Buffer is not required.

For detection with HRP or AP, different buffers and solutions are recommended. The composition is listed in the following tables.

| Recommended buffers and solutions for detection with HRP | Concentration |
|---|---|
| PBS buffer | 4 mM $KH_2PO_4$, pH 7.4<br>16 mM $Na_2HPO_4$<br>115 mM NaCl |
| PBS-blocking buffer | PBS buffer<br>3% BSA<br>0.05% v/v Tween 20 |
| PBS-Tween buffer | PBS buffer<br>0.1% Tween 20 |

HRP allows chemiluminescent and chromogenic detection. For chemiluminescent detection with HRP, enzyme dilution buffer (PBS buffer, 0.2% BSA, 0.1% Tween) and chemiluminescence detection solution (offered by several suppliers) are required. For chromogenic detection, chloronaphthol solution (3% w/v 4-chloro-1-naphthol in methanol) and $H_2O_2$ solution (30% v/v) are necessary.

| Recommended buffers and solutions for detection with AP | Concentration |
|---|---|
| TBS buffer | 50 mM Tris/HCl, pH 7.4<br>140 mM NaCl |
| TBS-blocking buffer | TBS buffer<br>3% BSA<br>0.1% w/v Tween 20 |
| TBS-Tween buffer | TBS buffer<br>0.1% w/v Tween 20 |
| Reaction buffer | 100 mM Tris/HCl, pH 8.8<br>100 mM NaCl<br>5 mM MgCl2 |
| NTB solution | 7.5% w/v nitrotetrazolium blue in<br>70% v/v dimethylformamide |
| BCIP solution | 5% w/v 5-bromo-4-chloro-3-indolyl-phosphate in dimethyl-formamide |

# 3 PROTOCOL

## 3.1 Chemiluminescent detection with Strep-Tactin® HRP

**3.1.1** After SDS-PAGE and electro transfer to an appropriate membrane, block with 20 ml PBS-blocking buffer. Incubate for 1 h at room temperature or overnight at 4 °C while gently shaking.

**3.1.2** Wash 3 times with PBS-Tween buffer for 5 min at room temperature, gently shaking.

**3.1.3** After the last washing step, add 10 ml PBS-Tween buffer to the membrane.

**3.1.4** Optional: Before detection of Strep-tag® proteins, add 10 µl Biotin Blocking Buffer and incubate for 10 min at room temperature, gently shaking.

**3.1.5** Pre-dilute Strep-Tactin® HRP 1:100 in Enzyme dilution buffer. Add 10 µl to 10 ml PBS-Tween buffer. Incubate 1 h at room temperature, gently shaking.

**3.1.6** Wash 2 times with PBS-Tween buffer for 1 min at room temperature, gently shaking. Wash 2 more times with PBS buffer for 1 min at room temperature, gently shaking.

**3.1.7** Develop chemiluminescence reaction according to the instruction of your respective kit.

## 3.2 Chemiluminescent detection with StrepMAB-Classic HRP

**3.2.1** After SDS-PAGE and electrotransfer to an appropriate membrane, block with 20 ml PBS-blocking buffer. Incubate for 1 h at room temperature or overnight at 4 °C while gently shaking.

**3.2.2** Wash 3 times with PBS-Tween buffer for 5 min at room temperature, gently shaking.

**3.2.3** After the last washing step, add 10 ml PBS-Tween buffer to the membrane.

**3.2.4** Pre-dilute StrepMAB-Classic HRP conjugate 1:10 in Enzyme dilution buffer. Add 3 µl to 10 ml PBS-Tween buffer. When blocking with 1% milk powder in PBS-Tween buffer use 4 µl undiluted StrepMAB-Classic HRP conjugate instead. Incubate 1 h at room temperature, gently shaking.

**3.2.5** Wash 2 times with PBS-Tween buffer for 1 min at room temperature, gently shaking. Wash 2 more times with PBS buffer for 1 min at room temperature, gently shaking.

**3.2.6** Develop chemiluminescence reaction according to the instruction of your respective kit.

## 3.3 Chromogenic detection with Strep-Tactin® HRP

**3.3.1** After SDS-PAGE and electrotransfer to an appropriate membrane, block with 20 ml PBS-blocking buffer. Incubate for 1 h at room temperature or overnight at 4 °C while gently shaking.

**3.3.2** Wash 3 times with PBS-Tween buffer for 5 min at room temperature, gently shaking.

**3.3.3** After the last washing step, add 10 ml PBS-Tween buffer to the membrane.

**3.3.4** Optional: Before detection of Strep-tag® proteins, add 10 µl Biotin Blocking Buffer and incubate for 10 min at room temperature, gently shaking.

**3.3.5** Directly add 2.5 µl Strep-Tactin® HRP (1:4000). Incubate 60 min at room temperature, gently shaking

**3.3.6** Wash 2 times with PBS-Tween buffer for 1 minute at room temperature, gently shaking. Wash 2 more times with PBS-buffer for 1 min at room temperature, gently shaking.

**3.3.7** Transfer membrane in 20 ml PBS buffer, add 200 µl chloronaphthol solution and 20 µl $H_2O_2$ solution.

**3.3.8** Perform the chromogenic reaction under shaking. Stop reaction by washing several times with distilled $H_2O$. Air dry the membrane and store it in the dark.

### 3.4 Chromogenic detection with StrepMAB-Classic HRP

**3.4.1** After SDS-PAGE and electrotransfer to an appropriate membrane, block with 20 ml PBS-blocking buffer. Incubate for 1 h at room temperature or overnight at 4 °C while gently shaking.

**3.4.2** Wash 3 times with PBS-Tween buffer for 5 min at room temperature, gently shaking.

**3.4.3** After the last washing step, add 10 ml PBS-Tween buffer to the membrane.

**3.4.4** Directly add 2.5 µl StrepMAB-Classic HRP. Incubate 60 min at room temperature, gently shaking.

**3.4.5** Wash 2 times with PBS-Tween buffer for 1 min at room temperature, gently shaking. Wash 2 more times with PBS-buffer for 1 min at room temperature, gently shaking.

**3.4.6** Transfer membrane in 20 ml PBS buffer, add 200 µl chloronaphthol solution and 20 µl $H_2O_2$ solution.

**3.4.7** Perform the chromogenic reaction under shaking. Stop reaction by washing several times with distilled $H_2O$. Air dry the membrane and store it in the dark.

### 3.5 Chromogenic detection with Strep-Tactin® AP

**3.5.1** After SDS-PAGE and electrotransfer to an appropriate membrane, block with 20 ml TBS-blocking buffer. Incubate for 1 h at room temperature or overnight at 4 °C while gently shaking.

**3.5.2** Wash 3 times with TBS-Tween buffer for 5 min at room temperature, gently shaking.

**3.5.3** After the last washing step, add 10 ml TBS-Tween buffer to the membrane.

**3.5.4** Optional: Before detection of with Strep-Tactin® AP add 10 µl Biotin Blocking Buffer and incubate for 10 min at room temperature, gently shaking.

**3.5.5** Add 2.5 µl Strep-Tactin® AP (1:4000). Incubate 1 h at room temperature, gently shaking.

**3.5.6** Wash 2 times with TBS-Tween buffer for 1 minute at room temperature, gently shaking. Wash 2 more times with TBS buffer for 1 min at room temperature, gently shaking.

**3.5.7** Transfer membrane in 20 ml reaction buffer and add 10 µl NBT solution and 60 µl BCIP solution. Proceed with the chromogenic reaction under shaking until optimal signal to background ratio is achieved. Stop reaction by washing several times with distilled $H_2O$. Air dry the membrane and store in the dark.

# 4 TROUBLESHOOTING

## 4.1 No signal

| | |
|---|---|
| Not enough StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP is bound to the protein of interest. | Use more concentrated StrepMAB-Classic or Strep-Tactin®. Incubate longer (e.g., overnight) at 4 °C. |
| Insufficient antigen. | Load at least an aggregate of 20-30 µg protein per lane. Use protease inhibitors and a positive control. |
| 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.). |
| Poor transfer of protein to membrane. | Check the transfer with a reversible stain such as Ponceau S. Check that the transfer was not performed the wrong way. If using PVDF membrane, make sure you pre-soak the membrane in MeOH instead of transfer buffer. |
| Excessive washing of the membrane. | Do not over wash the membrane. |
| 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 PBS for 1 h. |
| StrepMAB-Classic HRP or Strep-Tactin® HRP inhibited by sodium azide. | Do not use sodium azide together with HRP conjugates. |
| Detection kit is old, and substrate is inactive. | Use fresh substrate. |

## 4.2 High background

| | |
|---|---|
| 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 PBS for 1 h. These can be included in the antibody buffers as well. |
| Concentration of StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP may be too high. | Titrate the antibody to the optimal concentration, incubate for longer but in more dilute antibody. |
| Incubation temperature may be too high. | Incubate blot at 4 °C. |
| Washing of unbound StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP may be insufficient. | Increase the number of washes. |
| Your choice of membrane may give high background. | Nitrocellulose membrane is considered to give less background than PVDF. |
| The membrane has dried out. | Care should be taken to prevent the membrane from drying out during incubation. |

### 4.3 Multiple bands

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|---|---|
| Cell lines that have been frequently passaged gradually accumulate differences in their protein expression profiles. | Go back to the original non-passaged cell line and run the current and original cell line samples in parallel. |
| The protein sample has multiple modified forms in vivo such as acetylation, methylation, myristylation, phosphorylation, glycosylation etc. | Examine the literature and use an agent to dephosphorylate, de-glycosylate, etc. the protein to bring it to the correct size. |
| The target in your protein sample has been digested (more likely if the bands are of lower molecular weight). | Make sure that you incorporate sufficient protease inhibitors in your sample buffer. |
| Concentration of StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP is too high - at high concentration multiple bands are often seen. | Try decreasing the antibody concentration and/or the incubation period. |
| The protein target may form multimers. | Try boiling in SDS-PAGE sample buffer for 10 minutes rather than 5 minutes to disrupt multimers. |

### 4.4 Uneven white “spots” on the blot

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|---|---|
| Air bubbles were trapped against the membrane during transfer or StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP is not evenly spread on the membrane. | Make sure you remove bubbles when preparing the gel for transfer. Incubate StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP under agitation. |

### 4.5 Black dots on the blot

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|---|---|
| StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP are binding to the blocking agent. | Filter the blocking agent. |

### 4.6 White bands on a black blot (negative of expected blot)

| | |
|---|---|
| Too much StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP | Dilute StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP more. |

### 4.7 MW marker lane is black

| | |
|---|---|
| StrepMAB-Classic HRP, or Strep-Tactin® HRP or Strep-Tactin® AP is reacting with the MW marker. | Add a blank lane between the MW marker and the first sample lane. |

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If you have any questions, please contact

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We are here to help!