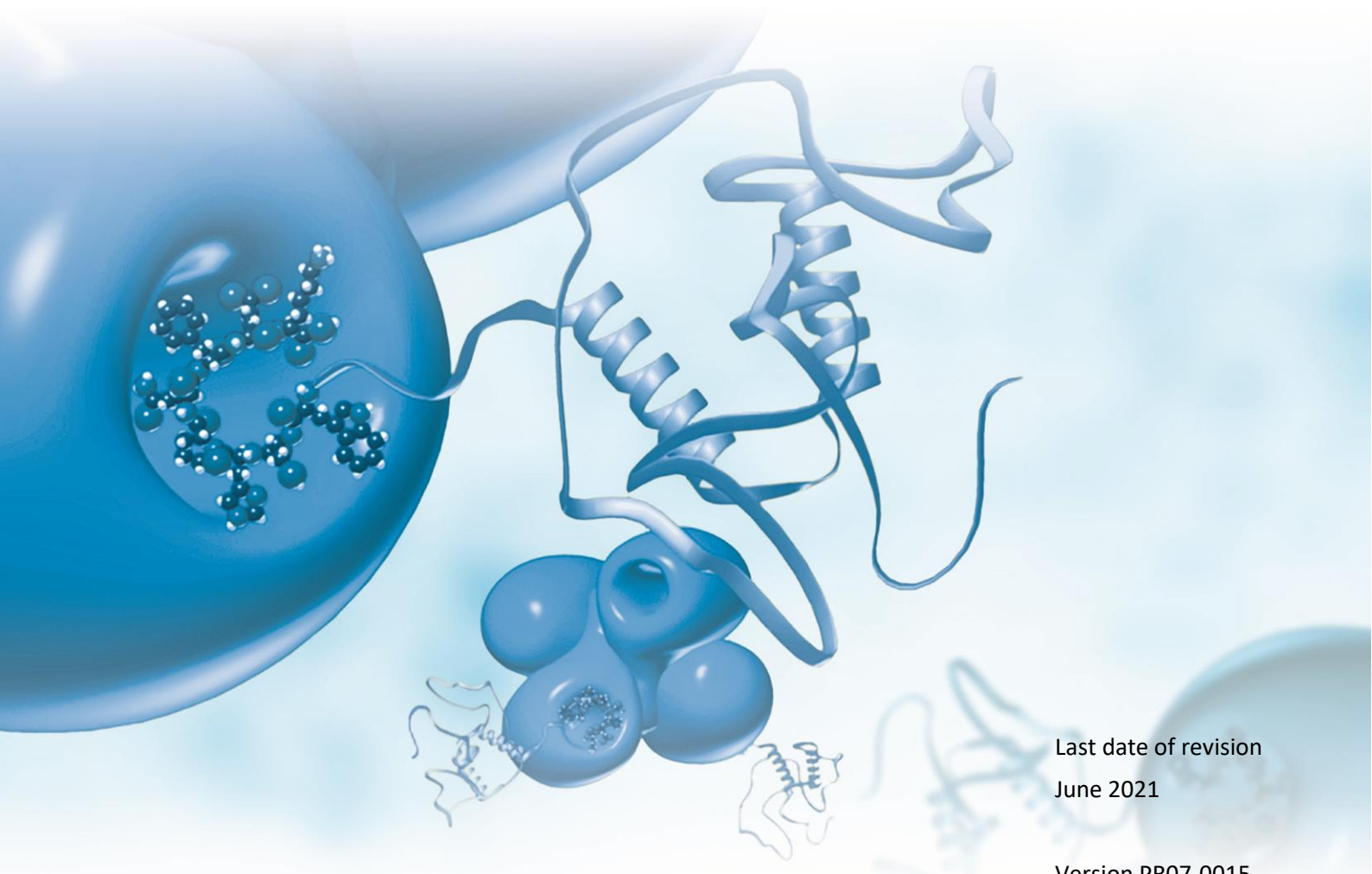


***Strep-tag*[®] detection in Western blots**

General protocol for the detection of *Strep-tag*[®] fusion proteins



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1 *Strep-Tactin*[®]-based *Strep-tag*[®] detection

1.1 Chemiluminescence detection with horse radish peroxidase (HRP)

Recommended Buffers/Solutions	Concentration of ingredients
PBS buffer	4 mM KH ₂ PO ₄ , pH 7.4 16 mM Na ₂ HPO ₄ 115 mM NaCl
PBS-blocking buffer	PBS buffer with 3% BSA and 0.05% v/v Tween 20
Enzyme dilution buffer	PBS with 0.2% BSA and 0.1% Tween
PBS-Tween buffer	PBS buffer with 0.1% v/v Tween 20
Chemiluminescence detection solution	



Important notes

- For blocking biotinylated proteins use Biotin Blocking Buffer (Cat. no. 2-0501-002)

Protocol

1. **After SDS-PAGE and electrotransfer of the protein to an appropriate membrane block the membrane with 20 ml PBS-blocking buffer. Incubate 1 h (room temperature; with gentle shaking) or overnight (4°C).**
We recommend to use a nitrocellulose membrane. Do not block with skimmed milk, since this contains Biotin which would be detected by *Strep-Tactin*[®] HRP and therefore produces high background.
2. **Wash 3 times with 20 ml PBS-Tween buffer (each step: 5 minutes, room temperature, gentle shaking).**
3. **After the last washing step, add 10 ml PBS-Tween buffer to the membrane.**
4. **Optional: Before detection of *Strep-tag*[®] proteins (step 5) add 10 µl Biotin Blocking Buffer (10 minutes, room temperature, gentle shaking).**
This blocks endogenously biotinylated proteins (e.g. the biotin carboxyl carrier protein (BCCP, 22 kDa) in case of *E. coli*) which will otherwise stain sensitively.
5. **Pre-dilute *Strep-Tactin*[®] horse radish peroxidase conjugate 1:100 in Enzyme dilution buffer (PBS, BSA, Tween) and add 10 µl to 10 ml PBS-Tween. Incubate 60 minutes at room temperature, gentle shaking.**
6. **Wash 2 times with PBS-Tween buffer (each step: 1 minute, room temperature, gentle shaking).**

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Protocol (continued)	<p>7. Wash 2 times with PBS-buffer (each step: 1 minute, room temperature, gentle shaking).</p> <p>8. Develop chemoluminescence reaction according to the instruction of your respective kit.</p>
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1.2 Chromogenic detection with horse radish peroxidase (HRP)

Recommended Buffers/Solutions	Concentration of ingredients
PBS buffer	4 mM KH ₂ PO ₄ , pH 7.4 16 mM Na ₂ HPO ₄ 115 mM NaCl
PBS-blocking buffer	PBS buffer with 3% BSA and 0.05% v/v Tween 20
PBS-Tween buffer	PBS buffer with 0.1% v/v Tween 20
Chloronaphtol solution	3% w/v 4-chloro-1-naphtol in methanol
H ₂ O ₂ solution	30% v/v H ₂ O ₂

Important notes

- For blocking biotinylated proteins use Biotin Blocking Buffer (Cat. no. 2-0501-002)

Protocol

1. **After SDS-PAGE and electrotransfer of the protein to an appropriate membrane block the membrane with 20 ml PBS-blocking buffer. Incubate: 1 h (room temperature; with gentle shaking) or overnight (4°C).**
We recommend to use a nitrocellulose membrane. Do not block with skimmed milk, since this contains Biotin which would be detected by *Strep-Tactin*[®] HRP and therefore produces high background.
2. **Wash 3 times with 20 ml PBS-Tween buffer (each step: 5 minutes, room temperature, gentle shaking).**
3. **After the last washing step, add 10 ml PBS-Tween buffer to the membrane.**
4. **Optional: Before detection *Strep-tag*[®] proteins (step 5) add 10 µl Biotin Blocking Buffer (10 minutes, room temperature, gentle shaking).**
This blocks endogenously biotinylated proteins (e.g. the biotin carboxyl carrier protein (BCCP, 22 kDa) in case of *E. coli*) which will otherwise stain sensitively.
5. **Add 2.5 µl *Strep-Tactin*[®] horse radish peroxidase conjugate (1:4000). Incubate 60 minutes at room temperature, gentle shaking.**

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Protocol (continued)	<ol style="list-style-type: none"> 6. Wash 2 times with PBS-Tween buffer (each step: 1 minute, room temperature, gentle shaking). 7. Wash 2 times with PBS-buffer (each step: 1 minute, room temperature, gentle shaking). 8. Transfer membrane in 20 ml PBS buffer, add 200 µl chloronaphthol solution and 20 µl H₂O₂ solution. 9. Proceed the chromogenic reaction under shaking until optimal signal:background ratio is achieved. 10. Stop reaction by washing several times with distilled H₂O. 11. Air dry the membrane and store it in the dark.
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1.3 Chromogenic detection with Alkaline Phosphatase (AP)

Recommended Buffers/Solutions	Concentration of ingredients
TBS buffer	50 mM Tris·HCl, pH 7.4 140 mM NaCl
TBS-blocking buffer	TBS buffer with 3% BSA and 0.1% w/v Tween 20
TBS-Tween buffer	TBS buffer with 0.1% w/v Tween 20
Reaction buffer	100 mM Tris·HCl, pH 8.8 100 mM NaCl 5 mM MgCl ₂
NTB solution	7.5% w/v nitrotetrazolium blue in 70% v/v dimethylformamid
BCIP solution	5% w/v 5-bromo-4-chloro-3-indolyl-phosphate in dimethyl-formamid



Important notes

- For blocking biotinylated proteins use Biotin Blocking Buffer (Cat. no. 2-0501-002)

Protocol

1. After SDS-PAGE and electrotransfer of the protein to an appropriate membrane block the membrane with 20 ml TBS-blocking buffer. Incubate 1 h (room temperature; with gentle shaking) or overnight (4°C).
We recommend to use a nitrocellulose membrane. Do not block with skimmed milk, since this contains Biotin which would be detected by *Strep-Tactin*[®] HRP and therefore produces high background.

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**Protocol
(continued)**

2. **Wash 3 times with 20 ml TBS-Tween buffer (each step: 5 minutes, room temperature, gentle shaking).**
3. **After the last washing step, add 10 ml TBS-Tween buffer to the membrane.**
4. **Optional: Before detection of *Strep*-tag[®] proteins (step 6) add 10 μ l Biotin Blocking Buffer (10 minutes, room temperature, gentle shaking).**
This blocks endogenously biotinylated proteins (e.g. the biotin carboxyl carrier protein (BCCP, 22 kDa) in case of *E. coli*) which will otherwise stain sensitively.
5. **Add 2.5 μ l *Strep*-Tactin[®] alkaline phosphatase conjugate (1:4000). Incubate 60 minutes at room temperature, gentle shaking.**
6. **Wash 2 times with TBS-Tween buffer (each step: 1 minute, room temperature, gentle shaking).**
7. **Wash 2 times with TBS-buffer (each step: 1 minute, room temperature, gentle shaking).**
8. **Transfer membrane in 20 ml reaction buffer and add 10 μ l NBT solution and 60 μ l BCIP solution.**
9. **Proceed with the chromogenic reaction under shaking until optimal signal:background ratio is achieved.**
10. **Stop reaction by washing several times with distilled H₂O.**
11. **Air-dry the membrane and store it in the dark.**



2 *Strep*MAB-Classic (Antibody)-based *Strep*-tag[®] detection

2.1 Chemiluminescence detection with horse radish peroxidase (HRP)

Recommended Buffers/Solutions	Concentration of ingredients
PBS buffer	4 mM KH ₂ PO ₄ , pH 7.4 16 mM Na ₂ HPO ₄ 115 mM NaCl
PBS-blocking buffer	PBS buffer with 3% BSA and 0.05% v/v Tween 20
Enzyme dilution buffer	PBS with 0.2% BSA and 0.1% Tween
PBS-Tween buffer	PBS buffer with 0.1% v/v Tween 20
Chemiluminescence detection solution	

Protocol	
	<ol style="list-style-type: none"> 1. After SDS-PAGE and electrotransfer of the protein to an appropriate membrane block the membrane with 20 ml PBS-blocking buffer (PBS buffer with 3% BSA and 0.05% v/v Tween 20). Incubate 1 h (room temperature; with gentle shaking) or overnight (4°C). If you want to use milk powder for blocking, we recommend 1 % milk powder in PBS with 0.1 % Tween. We recommend the use of nitrocellulose membrane. 2. Wash 3 times with 20 ml PBS-Tween buffer (each step: 5 minutes, room temperature, gentle shaking). 3. After the last washing step, add 10 ml PBS-Tween buffer to the membrane. 4. Pre-dilute <i>Strep</i>MAB-Classic horse radish peroxidase conjugate 1:10 in Enzyme dilution buffer (PBS, 0.2% BSA, 0.1% Tween) and add 3 µl to 10 ml PBS-Tween (when blocking with 1% milk powder in PBS-Tween use 4 µl undiluted <i>Strep</i>MAB-Classic horse radish peroxidase conjugate instead). Incubate 60 minutes at room temperature, gentle shaking. 5. Wash 2 times with PBS-Tween buffer (each step: 1 minute, room temperature, gentle shaking). 6. Wash 2 times with PBS-buffer (each step: 1 minute, room temperature, gentle shaking). 7. Develop chemiluminescence reaction according to the instruction of your respective kit.



2.2 Chromogenic detection with *Strep*MAB-Classic HRP

Recommended Buffers/Solutions	Concentration of ingredients
PBS buffer	4 mM KH ₂ PO ₄ , pH 7.4 16 mM Na ₂ HPO ₄ 115 mM NaCl
PBS-blocking buffer	PBS buffer with 3% BSA and 0.05% v/v Tween 20
PBS-Tween buffer	PBS buffer with 0.1 % v/v Tween 20
Chloronaphtol solution	3% w/v 4-chloro-1-naphtol in methanol
H ₂ O ₂ solution	30% v/v H ₂ O ₂

Protocol	
	<ol style="list-style-type: none"> 1. After SDS-PAGE, perform electrotransfer of the protein to an appropriate membrane. We recommend to use a nitrocellulose membrane. 2. Block the membrane with 20 ml PBS-blocking buffer. Incubate 1 h (room temperature; with gentle shaking) or overnight (4°C). 3. Wash 3 times with 20 ml PBS-Tween buffer (each step: 5 minutes, room temperature, gentle shaking). 4. After the last washing step, add 10 ml PBS-Tween buffer to the membrane. 5. Add 2.5 µl <i>Strep</i>MAB-Classic HRP conjugate. 6. Incubate 60 minutes at room temperature, gentle shaking. 7. Wash 2 times with PBS-Tween buffer (each step: 1 minute, room temperature, gentle shaking). 8. Wash 2 times with PBS buffer (each step: 1 minute, room temperature, gentle shaking). 9. Transfer membrane in 20 ml PBS buffer, add 200 µl chloronaphtol solution and 20 µl H₂O₂ solution. 10. Perform the chromogenic reaction under shaking. 11. Stop reaction by washing several times with distilled H₂O. 12. Air dry the membrane and store it in the dark.

3 Trouble shooting



3.1 No signal

The primary antibody and the secondary antibody are not compatible.	Use secondary antibody that was raised against the species in which the primary was raised (e.g. primary is raised in rabbit, use anti-rabbit secondary).
Not enough primary or secondary antibody is bound to the protein of interest.	Use more concentrated antibody. Incubate longer (e.g. overnight) at 4°C.
Cross-reaction between blocking agent and primary or secondary antibody.	Use a mild detergent such as Tween 20 or switch blocking reagent (i.e. commonly used blocking reagents are milk, BSA, serum or gelatin).
Insufficient antigen.	Load at least an aggregate of 20-30 µg protein per lane; use protease inhibitors; run the recommended 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 60 min.
Over-use of the primary antibody.	Use fresh antibody as the effective concentration is lowered upon each re-use.
Secondary antibody inhibited by sodium azide.	Do not use sodium azide together with HRP-conjugated antibodies.
Detection kit is old and substrate is inactive	Use fresh substrate.



3.2 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 60 min. These can be included in the antibody buffers as well.
The primary antibody concentration 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.
The secondary antibody may be binding non-specifically or reacting with the blocking reagent.	Run a secondary control without primary antibody.
Cross-reaction between blocking agent and primary or secondary.	Add a mild detergent such as Tween-20 to the incubation and washing buffer.
Washing of unbound antibodies 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.

3.3 Multiple bands

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.



3.3 Multiple bands (continued)

Primary antibody concentration is too high - at high concentration multiple bands are often seen.	Try decreasing the antibody concentration and/or the incubation period.
Secondary antibody concentration is too high - at high concentration secondaries will bind nonspecifically.	Try decreasing the concentration. Run a secondary antibody control (without the primary).
The protein target may form multimers.	Try boiling in SDS-Page for 10 minutes rather than 5 minutes to disrupt multimers.

3.4 Uneven white “spots” on the blot

Air bubbles were trapped against the membrane during transfer or the antibody is not evenly spread on the membrane.	Make sure you remove bubbles when preparing the gel for transfer. Incubate antibodies under agitation.
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3.5 Other Problems

3.5.1 Black dots on the blot

The antibodies are binding to the blocking agent.	Filter the blocking agent.
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3.5.2 White bands on a black blot (negative of expected blot)

Too much primary and/or too much secondary antibody.	Dilute the antibodies more.
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3.5.3 MW marker lane is black

The antibody is reacting with the MW marker.	Add a blank lane between the MW marker and the first sample lane.
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