Strep-tag® detection in Western blots

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

Last date of revision
July 2007

Version PR07-0009

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BioTAGnology

1. Chemiluminescence detection of *Strep*-tag proteins with *Strep*-Tactin horse radish peroxidase (HRP) conjugate

Material and important notes

- PBS buffer: 4 mM KH_2PO_4, 16 mM Na_2HPO_4, 115 mM NaCl, pH 7.4
- PBS-blocking buffer: PBS buffer with 3 % BSA and 0.5 % 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
- *Strep*-tag protein ladder can be used as positive control (cat. no. 2-1011-100)
- For blocking biotinylated proteins use Biotin Blocking Buffer (cat. no. 2-0501-002)
- Chemiluminescence detection solution

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.

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. **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).**

7. **Wash 2 times with PBS-buffer (each step: 1 minute, room temperature, gentle shaking).**

Develop chemolumininescence reaction according to the instruction of your respective kit.

2. Chemiluminescence detection of *Strep*-tag proteins with *Strep*MAB-Classic horse radish peroxidase (HRP) conjugate

Material and important notes

- PBS buffer: 4 mM KH_2PO_4, 16 mM Na_2HPO_4, 115 mM NaCl, pH 7.4
- PBS-blocking buffer: PBS buffer with 3 % BSA and 0.5 % v/v Tween 20
- PBS-Tween buffer: PBS buffer with 0.1 % v/v Tween 20
- Enzyme dilution buffer: PBS with 0.2% BSA and 0.1 % Tween
- *Strep*-tag protein ladder can be used as positive control (cat. no. 2-1011-100)
- Chemiluminescence detection solution

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 0.5 % 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. Optimal dilution of StrepMAB-Classic horse radish peroxidase conjugate should then be 1:4000 (instead of 1:30000).
   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 *Strep*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 *Strep*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).**

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

3. Chromogenic detection of *Strep*-tag proteins with *Strep*-Tactin Alkaline Phosphatase (AP) conjugate

Material and important notes

- PBS buffer: 4 mM KH_2PO_4, 16 mM Na_2HPO_4, 115 mM NaCl, pH 7.4
- PBS blocking buffer: PBS buffer with 3 % BSA and 0.5 % v/v Tween 20
- PBS-Tween buffer: PBS buffer with 0.1 % v/v Tween 20
- Reaction buffer: 100 mM NaCl, 5 mM $MgCl_2$, 100 mM Tris·Cl, pH 8.8
- 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
- *Strep*-tag protein ladder can be used as positive control (cat. no. 2-1011-100).
- For blocking biotinylated proteins use Biotin Blocking Buffer (cat. no. 2-0501-002).

1. **Perform SDS-PAGE and 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. **Optional: Before detection *Strep*-tag proteins (step 6) 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.

6. **Add 2.5 µl *Strep*-Tactin alkaline phosphatase conjugate (1:4000). 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 reaction buffer and add 10 µl NBT solution and 60 µl BCIP solution.**

10. **Proceed with the chromogenic reaction under shaking until optimal signal:background ratio is achieved.**

11. Stop reaction by washing several times with distilled H_2O.

12. Air dry the membrane and store it in the dark.

4. Chromogenic detection of *Strep*-tag proteins with *Strep*-Tactin horse radish peroxidase (HRP) conjugate

Material and important notes

- PBS buffer: 4 mM KH_2PO_4, 16 mM Na_2HPO_4, 115 mM NaCl, pH 7.4
- PBS-blocking buffer: PBS buffer with 3 % BSA and 0.5 % 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_2O_2 solution: 30 % v/v H_2O_2
- *Strep*-tag protein ladder can be used as positive control (cat. no. 2-1011-100).
- For blocking biotinylated proteins use Biotin Blocking Buffer (cat. no. 2-0501-002).

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.

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.**

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 chloronaphtol solution and 20 µl H_20_2 solution.**

9. **Proceed the chromogenic reaction under shaking until optimal signal:background ratio is achieved.**

10. **Stop reaction by washing several times with distilled H_2O.**

11. **Air dry the membrane and store it in the dark.**

5. Chromogenic detection of *Strep*-tag proteins with *Strep*MAB-Classic HRP conjugate

Material and important notes

- PBS buffer: 4 mM KH_2PO_4, 16 mM Na_2HPO_4, 115 mM NaCl, pH 7.4
- PBS blocking buffer: PBS buffer with 3 % BSA and 0.5 % 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_2O_2 solution: 30 % v/v H_2O_2
- *Strep*-tag protein ladder can be used as positive control (cat. no. 2-1011-100).

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 *Strep*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 H_20_2 solution.**

10. **Perform the chromogenic reaction under shaking.**

11. **Stop reaction by washing several times with distilled H_2O.**

12. **Air dry the membrane and store it in the dark.**

6. Trouble shooting

6.1 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 then in 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 0.5 % 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.

6.2 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 0.5 % v/v Tween 20 in PBS for 60 min. These can be included in the antibody buffers as well. **An alternative protocol can be provided, please inquire.**
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.

6.3 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.

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 bands may be non-specific.	Where possible use blocking peptides to differentiate between specific and non-specific bands. Only specific bands should be blocked (and thus disappear).
The protein target may form multimers.	Try boiling in SDS-Page for 10 minutes rather than 5 minutes to disrupt multimers.

6.4 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.

6.5 Other Problems

6.5.1 Black dots on the blot

The antibodies are binding to the blocking agent.	Filter the blocking agent.

6.5.2 White bands on a black blot (negative of expected blot)

Too much primary and/or too much secondary antibody.	Dilute the antibodies more.

6.5.3 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.

For research use only

Strep-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and *Strep*-Tactin® is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use. *Strep*-tag® and *Strep*-Tactin® are registered trademarks of IBA GmbH.