Strep-Tactin AP conjugate Western Blot

Snehadri Sinha

Abstract

Protocol for western blot to detect strep-tagged constructs, using chromogenic detection with Strep-Tactin AP (Alkaline Phosphatase) conjugate. Based on protocol from iba life sciences.

Citation: Snehadri Sinha Strep-Tactin AP conjugate Western Blot. **protocols.io**
dx.doi.org/10.17504/protocols.io.gznbx5e
Published: 17 Jan 2017

Protocol

Blotting

Step 1.
Run your protein samples on SDS-PAGE as usual

Step 2.
Soak 6 pieces of Whatman blotting paper, nitrocellulose membrane (all a bit bigger than your gel) and your SDS-PAGE gel in transfer buffer for a few minutes

Step 3.
Assemble the blot: 3 layers of Whatman paper, nitorcellulose membrane (to the positive pole of the device; in the Bio-Rad blotting machine to the bottom), SDS-PAGE gel, 3 layers of Whatman paper

Step 4.
Roll out all possible air bubbles with a tube or some other device

Step 5.
Blot, use appropriate settings according to your blotting machine.

Chromogenic detection wit

Step 6.
Block the membrane with 20 mL TBS-blocking buffer: incubate 1 h at RT with gentle shaking or O/N at 4 C.

Step 7.
Wash 3 times with 20 mL TBS-Tween buffer (each step: 5 min, RT, gentle shaking)

Step 8.
After the last washing step, add 10 mL TBS-Tween buffer to the membrane

Step 9.
(Optional: add 10 uL Biotin Blocking Buffer: 10 min, RT, shaking - blocks endgenously biotinylated proteins which could stain sensitively)

Step 10.
Add 2.5 uL Strep-Tactin AP conjugate (1:4000). Incubate 60 minutes at RT with gentle shaking.

Step 11.
Wash 2 times with TBS-Tween buffer (each step: 1 min, RT, gentle shaking)

Step 12.
Wash 2 times with TBS-buffer (each step: 1 min, RT, gentle shaking)

Step 13.
Transfer membrane in 20 mL reaction buffer with NBT and BCIP. Use manufacturer's instructions for NBT and BCIP amounts; e.g. for Promega NBT+BCIP, first add NBT (33uL for each 5 mL of reaction buffer), mix, add BCIP (16.5 uL for each 5 mL of reaction buffer), mix again, then use within 1 hour)

Step 14.
Proceed with chromogenic reaction under shaking until optimal signal:background ratio is acheived.

Step 15.
Stop reaction by washing several times with distilled H2O.

Step 16.
Air-dry the membrane and store it in the dark.