JNK2 (56G8) Rabbit mAb #9258

Protocol

Western Blotting Protocol

For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

NOTE: Please refer to primary antibody datasheet or product webpage for recommended antibody dilution.

A. Solutions and Reagents

From sample preparation to detection, the reagents you need for your Western Blot are now in one convenient kit: #12957 (/product/productDetail.jsp?productId=12957) Western Blotting Application Solutions Kit

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS)**: (#9808 (/product/productDetail.jsp?productId=9808)) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS)**: (#12498 (/product/productDetail.jsp?productId=12498)) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer**: Blue Loading Pack (#7722 (/product/productDetail.jsp?productId=7722)) or Red Loading Pack (#7723 (/product/productDetail.jsp?productId=7723)) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer**: (#4050 (/product/productDetail.jsp?productId=4050)) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer**: (#12539 (/product/productDetail.jsp?productId=12539)) To prepare 1 L 1X Transfer Buffer: add 100 ml 10X Transfer Buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST)**: (#9997 (/product/productDetail.jsp?productId=9997)) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk**: (#9999 (/product/productDetail.jsp?productId=9999)).
8. **Blocking Buffer**: 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer**: (#9997 (/product/productDetail.jsp?productId=9997)) 1X TBST.
10. **Bovine Serum Albumin (BSA)**: (#9998 (/product/productDetail.jsp?productId=9998)).
11. **Primary Antibody Dilution Buffer**: 1X TBST with 5% BSA; for 20 ml, add 1.0 g BSA to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack**: (#7727 (/product/productDetail.jsp?productId=7727)).
13. **Prestained Protein Marker, Broad Range (11-190 kDa)**: (#13953 (/product/productDetail.jsp?productId=13953)).
14. **Blotting Membrane and Paper**: (#12369 (/product/productDetail.jsp?productId=12369)) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP**: Anti-rabbit IgG, HRP-linked Antibody (#7074 (/product/productDetail.jsp?productId=7074)).
16. **Detection Reagent**: SignalFire™ ECL Reagent (#6883 (/product/productDetail.jsp?productId=6883)).

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on

ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

   NOTE: Loading of prestained molecular weight markers (#13953 (/product/productDetail.jsp?productId=13953), 5 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727 (/product/productDetail.jsp?productId=7727), 10 µl/lane) to determine molecular weights are recommended.

8. Electrotransfer to nitrocellulose membrane (#12369 (/product/productDetail.jsp?productId=12369)).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with Anti-rabbit IgG, HRP-linked Antibody (#7074 (/product/productDetail.jsp?productId=7074) at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 (/product/productDetail.jsp?productId=7075) at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

Directions for Use:

1. Wash membrane-bound HRP (antibody conjugate) three times for 5 minutes in TBST.
2. Prepare 1X SignalFire™ ECL Reagent (#6883 (/product/productDetail.jsp?productId=6883)) by diluting one part 2X Reagent A and one part 2X Reagent B (e.g. for 10 ml, add 5 ml Reagent A and 5 ml Reagent B). Mix well.
3. Incubate substrate with membrane for 1 minute, remove excess solution (membrane remains wet), wrap in plastic and expose to X-ray film.

* Avoid repeated exposure to skin.

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Western Blot Reprobing Protocol

Reprobing of an existing membrane is a convenient means to immunoblot for multiple proteins independently when only a limited amount of sample is available. It should be noted that for the best possible results a fresh blot is always recommended. Reprobing can be a valuable method but with each reprobing of a blot there is potential for increased background signal. Additionally, it is recommended that you verify the removal of the first antibody complex prior to reprobing so that signal attributed to binding of the new antibody is not leftover signal from the first immunoblotting experiment. This can be done by re-exposing the blot to ECL reagents and making sure there is no signal prior to adding the next primary antibody.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalently purified water.

1. **Wash Buffer:** Tris Buffered Saline with Tween® 20 (TBST-10X) (#9997 (/product/productDetail.jsp?productId=9997))
2. **Stripping Buffer:** To prepare 100 ml, mix 6.25 ml of 1M Tris-HCl pH 6.8, 10 ml of 20% SDS and 700 µl β-mercaptoethanol. Bring to 100 ml with deionized H_20. Make buffer fresh just prior to use.

B. Protocol

1. After film exposure, wash membrane four times for 5 min each in TBST. Best results are obtained if the membrane is not allowed to dry.
2. Incubate membrane for 30 min at 50°C in stripping buffer (with slight agitation).
3. Wash membrane six times for 5 min each in TBST.
4. (Optional) To assure that the original signal is removed, wash membrane twice for 5 min each with 10 ml of TBST. Incubate membrane with LumiGLO® with gentle agitation for 1 min at room temperature. Drain membrane of excess developing solution. Do not let dry. Wrap in plastic wrap and expose to x-ray film.
5. Wash membrane again four times for 5 min each in TBST.
6. The membrane is now ready to reuse. Start detection at the "Membrane Blocking and Antibody Incubations" step in the Western Immunoblotting Protocol.

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