Anti-ACTIVE® MAPK, p38 and JNK Polyclonal Antibodies and Anti-ACTIVE® Qualified Secondary Ab Conjugate

INSTRUCTIONS FOR USE OF PRODUCTS V1211,V8031,V7931,V7932 AND V7951

Quick PROTOCOL

Western Blot Analysis with Anti-ACTIVE® pAbs

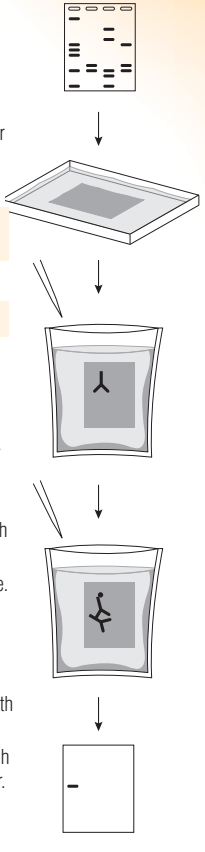
1. Perform SDS-PAGE analysis, and then transfer to either **nitrocellulose** or **PVDF** membrane.
2. Block the membrane. If using **nitrocellulose** membrane, block with TBS buffer/1% BSA for 1 hour at 37°C or overnight at 4°C. If using **PVDF** membrane, block with PVDF buffer (TBS/0.2% I-Block*/0.1% Tween-20) for 1 hour at 37°C or overnight at 4°C.
3. Prepare the primary antibody for incubation with the membrane.

Recommended Dilutions for the Anti-ACTIVE Abs

Antibody	Catalog #	Dilution
Anti-ACTIVE® MAPK	V8031	1:5000
Anti-ACTIVE® JNK	V7931, V7932	1:5000
Anti-ACTIVE® p38	V1211	1:2000

For **nitrocellulose** membranes, add the Anti-ACTIVE® pAb, diluted with TBST/0.1% BSA, and incubate 2 hours at room temperature with agitation. For **PVDF** membranes, add the Anti-ACTIVE® pAb, diluted with PVDF buffer and incubate as described for nitrocellulose membranes.

4. Wash each membrane 3 times. For **nitrocellulose** membranes, wash with 75ml of TBST buffer, 15 minutes for each wash. For **PVDF** membranes, wash using 75ml PVDF buffer, 15 minutes for each wash.
5. Prepare the secondary antibody conjugate for incubation with the membrane. The recommended dilution for the Anti-ACTIVE® Qualified Donkey Anti-Rabbit antibody conjugate, HRP is 1:5000 to 1:10,000.** For **nitrocellulose** membranes, dilute the Donkey Anti-Rabbit conjugate with TBST/0.1% BSA and incubate one hour at room temperature with agitation. For **PVDF** membranes, dilute the Donkey Anti-Rabbit Antibody conjugate with PVDF buffer and incubate as described for nitrocellulose membranes.
6. For **nitrocellulose** membranes, wash the membrane 3 times, 15 minutes each in 75ml TBST buffer. Rinse membrane 2 times, 1 minute each, in TBS buffer. Decant the solution after each wash and after each rinse. For **PVDF** membranes, wash 3 times as above using PVDF buffer. Rinse 2 times, 1 minute each, in TBS buffer. Decant solutions as described for nitrocellulose membranes.
7. Detect using the appropriate chemiluminescent or colorimetric protocol based on the secondary antibody conjugate used in Step 5.



Perform SDS-PAGE. Transfer gel to membrane.

Block membrane.

Incubate membrane with diluted primary antibody with agitation for 2 hours. Wash 3 times for 15 minutes each.

Incubate membrane with diluted secondary antibody. Wash 3 times for 15 minutes each time. Rinse 2 times for 1 minute each time.

Detect with protocol appropriate for the secondary antibody used.

*I-Block is a highly purified preparation of casein, prequalified for use with Western Blotting applications. I-Block is a trademark of Tropix, Inc.
**Dilute AP secondary antibody conjugates as recommended by manufacturer.
TBS Buffer: 20mM Tris-HCl (pH 7.5), 150mM NaCl TBST: Tbs Buffer with 0.05% Tween® 20

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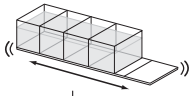
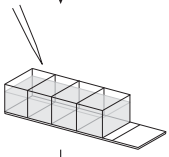
Quick PROTOCOL

Immunocytochemistry Protocol for Anti-ACTIVE® pAbs

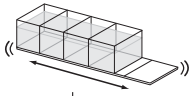
The following method outlines immunostaining of PC12 cells stimulated by either nerve growth factor (NGF) to activate MAP Kinases or by sorbitol to activate JNK and p38 kinases. Cells should be grown, prepared and activated as described in Technical Bulletin #TB262.



Wash activated cells once with cold PBS.

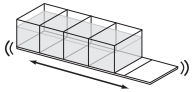


Fix cells with 10% paraformaldehyde, for 30 minutes at room temperature.

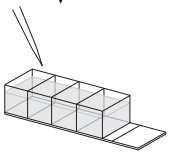


Wash 3 times for 5 minutes each in PBS.

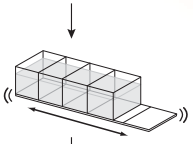
Permeabilize the cells with –20° C methanol for 10 minutes.



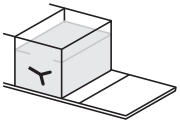
Wash 3 times for 5 minutes each in PBS.



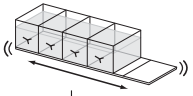
Incubate with blocking buffer for 3 hours at room temperature.



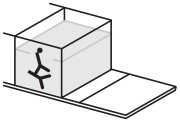
Wash once with PBS for 5 minutes.



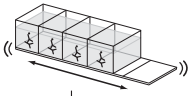
Incubate with diluted primary antibody overnight at 4°C.



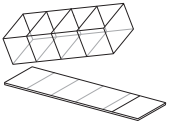
Wash 5 times for 15 minutes each in PBS.



Incubate with diluted secondary antibody for 90 minutes at room temperature.



Wash 5 times for 15 minutes each in PBS.



Remove grid and mount slides.

(continued in next column)

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