NEUROMICS

p38α Data Sheet

Catalog Number:	RA15053	**Host:**	Rabbit
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human, Rat, Mouse
Immunogen Sequence:	Purified, *E. coli*-derived recombinant human p38 alpha mitogen-activated protein kinase (MAPK14) alpha (GenBank accession # Q16539).	**Format:**	Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.
		Reconstitution:	Reconstitute in 100 µL of PBS containing 0.02% NaN3.

Applications: Western Blot-0.1 - 0.25 µg/mL
Immunohistochemistry-1.7 - 5 µg/mL.

Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Antibody can be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. *Avoid repeated freeze-thaw cycles.*

Application Notes

Specificity
The antibody detects endogenous human, mouse and rat p38α. The antibody does not detect recombinant p38β, p38γ or p38δ.

Immunohistochemistry
This antibody will detect p38α in cells and tissues. The working dilution is 1.7 - 5 µg/mL.

Western Blot
Protocol

Blotting Buffer
25 mM Tris, pH 7.4
0.15 M NaCl
0.1% Tween® 20

Blocking Solution
5% nonfat dry milk in blotting buffer
Adjust pH to 7.4

Antibody Solution
5% nonfat dry milk in blotting buffer
Adjust pH to 7.4

1. Transfer the electrophoresed proteins to Immobilon filters (Millipore) and incubate the membrane for 1 hour at room temperature in blocking solution.
2. Incubate the membrane overnight at 4° C in antibody solution containing 0.1 - 0.25 µg/mL rabbit anti human/rat/mouse p38α.
3. Wash the membrane at room temperature for 1 hour with 5 or more changes of blotting buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in antibody solution containing a 1:2,000 dilution of HRP-conjugated anti-rabbit IgG (Amersham).

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5. Wash the membrane for 1 hour with 5 or more changes of blotting buffer.

6. Detect with ECL Reagent (Amersham).

Cell lysates for Western blottings
To prepare total cell lysates, cells are solubilized in hot 2x SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, 10 mM NaF and bromophenyl blue) at 2 x 106 - 1 x 107 cells per mL. The extracts are heated in a boiling water bath for 5 minutes and then sonicated with a probe sonicator with 3 - 4 bursts of 5 - 10 seconds each. Samples are diluted with 1x SDS sample buffer to the desired concentration.

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