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<b>Catalog Number:</b>	RA15053	<b>Host:</b>	Rabbit
<b>Product Type:</b>	Affinity Purified Antibody	<b>Species Reactivity:</b>	Human, Rat, Mouse
<b>Immunogen Sequence:</b>	Purified, <i>E. coli</i> -derived recombinant human p38 alpha mitogen-activated protein kinase (MAPK14) alpha (GenBank accession # Q16539).	<b>Format:</b>	Lyophilized from a 0.2 $\mu$ m filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.
<b>Applications:</b>	Western Blot-0.1 - 0.25 $\mu$ g/mL Immunohistochemistry-1.7 - 5 $\mu$ g/mL.	<b>Reconstitution:</b>	Reconstitute in 100 $\mu$ L of PBS containing 0.02% NaN <sub>3</sub> .
<b>Storage:</b>	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator. 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. <i>Avoid repeated freeze-thaw cycles.</i>		

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### Application Notes

#### Specificity

The antibody detects endogenous human, mouse and rat p38 $\alpha$ . The antibody does not detect recombinant p38 $\beta$ , p38 $\gamma$  or p38 $\delta$ .

#### Immunohistochemistry

This antibody will detect p38 $\alpha$  in cells and tissues. The working dilution is 1.7 - 5  $\mu$ g/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  $\mu$ g/mL rabbit anti human/rat/mouse p38 $\alpha$ .
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  $2 \times 10^6$  -  $1 \times 10^7$  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.

Tween is a registered trademark of ICI Americas.

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