



PRAK, active
Human, Recombinant

Product Number **P 0365**

Synonyms: P38-Regulated/Activated Protein Kinase

Product Description

PRAK is a 471 amino acid serine/threonine kinase whose activity is strictly regulated by p38 α and p38 β (members of the MAP kinase superfamily).^{1,2,3,4,5} PRAK shares 20-30% sequence identity to the known MAP kinase-regulated protein kinases RSK1/2/3, MNK1/2, and MAPKAP-K2/3. Like all known MAP kinase-regulated protein kinases, PRAK has a MAP kinase phosphorylation site located in the T-loop between kinase subdomain VII and VIII.¹ However, PRAK differs from RSK members in that it lacks a second N-terminal kinase domain.^{1,6}

PRAK is expressed in numerous human tissues and cell lines. In HeLa cells, for example, PRAK is activated in response to cellular stress and proinflammatory cytokines.¹ PRAK is regulated by p38 α and p38 β both in vitro and in vivo with its regulatory phosphorylation site being Thr182. PRAK, a stress-activated protein kinase, specifically phosphorylates small heat shock protein 27 (HSP27).¹

The entire family of p38 MAP kinases can be activated by osmotic changes in the extracellular environment.¹ Other stimuli include UV light, oxidation, proinflammatory cytokines, and some growth factors.^{7,8} SB 203580 is a specific inhibitor of p38 α and p38 β .^{9,10} Activation of PRAK in intact cells is thus blocked by SB 203580 which in turn blocks HSP27 phosphorylation.¹

The 54 kDa recombinant PRAK, expressed in Sf9 cells, contains the full length amino acid sequence for human PRAK with an N-terminal His-tag. It is purified using Ni-NTA agarose, activated by MAP Kinase, and the activator removed on GSH-agarose. The recombinant human PRAK also phosphorylates PRAK Substrate Peptide (P 0240).

Components

Supplied as a frozen solution in 50 mM Tris-HCl, pH 7.5, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 0.15 M NaCl, 0.27 M sucrose, and 0.02% Brij-35.

Product Information

Precautions and Disclaimer

For laboratory use only. Not for drug, household or other uses. Please consult Material Safety Data Sheet for handling recommendations.

Storage/Stability

Stable for six months from date of shipment when properly stored at -70°C .

Specific Activity

30-150 units/mg protein.

One unit will incorporate 1 nmol of phosphate into substrate peptide per minute at pH 7.2 at 30°C .

Protein Kinase Assay Procedure

Stock Solutions

1. Dilute PRAK Substrate Peptide (P 0240) to $0.17\ \mu\text{g}/\mu\text{l}$ in Assay Dilution Buffer (ADB) (20 mM MOPS, pH 7.2, 25 mM β -glycerol phosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 1 mM dithiothreitol). A final concentration of $30\ \mu\text{M}$ is used per assay. (10 μl /assay).
2. Dilute PRAK, active to 20 ng/ μl in ADB. A volume of 10 μl will be used per assay.
3. Prepare 10 μl aliquots of a 1mCi/100 μl stock of [γ - 32]ATP (100 μCi /vial). Before beginning the assay, dilute an aliquot to 1 $\mu\text{Ci}/\mu\text{l}$ with 90 μl of 75 mM MgCl_2 and 500 μM cold ATP.

Assay Procedure:

1. Add 20 μl ADB to a microcentrifuge tube.
2. Add 10 μl of $0.17\ \mu\text{g}/\mu\text{l}$ PRAK Substrate Peptide.
3. Add 10 μl of diluted PRAK, active (200ng purified enzyme per assay).
4. Add 10 μl of the diluted [γ - 32]ATP solution
5. Incubate with agitation for 10 minutes at 30°C .
6. Transfer a 35 μl aliquot onto the center of a 2 cm by 2 cm P81 paper square.
7. Wash the paper squares three times with 0.75% phosphoric acid.
8. Wash the paper squares once for 3 minutes with acetone.
9. Transfer the paper squares to a scintillation vial and add 5 ml scintillation cocktail.
10. Read in a scintillation counter.

References

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Related Products

H 8158	Heat Shock Protein, HSP 27
M 8057	P38 MAP Kinase, Non-Activated N-terminal Histidine-tagged
M 0800	Anti-p38 MAP Kinase
M 8177	Monoclonal Anti-p38 MAP Kinase, Activated (Diphosphorylated p38)
M 8432	Monoclonal Anti-p38 MAP Kinase, Non-Activated
M 4046	Mitogen Activated Protein Kinase 2 Fragment 326-339
P 0240	PRAK Substrate Peptide
S 8307	SB 203580

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