PRAK Substrate Peptide
Product Number **P 0240**

ProductInformation

Synonyms: P38 Regulated/Activated
Protein Kinase Substrate Peptide

Product Description

The 1171 dalton synthetic decapeptide, KKLRRTLSVA, is provided as a substrate for p38-Regulated/Activated Protein Kinase or PRAK, active (P 0365). PRAK is a 471 amino acid serine/threonine kinase that is activated in response to cellular stress and proinflammatory cytokines.[1,2,3] Activity of PRAK is regulated by p38α and p38β (members of the MAP kinase superfamily). Activated PRAK will phosphorylate PRAK Substrate Peptide and small heat shock protein 27 (HSP27).[1]

Supplied as a frozen solution in water.

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

Undiluted solution is stable for one year upon delivery if properly stored at -20 °C. Aliquot to avoid repeated freeze-thaw cycles.

Protein Kinase Assay Procedure

Stock Solutions

1. Dilute PRAK Substrate Peptide to 0.17 µg/µl 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 µM is used per assay (10 µl/assay).
2. Dilute PRAK, active (P0365) to 20 ng/µl in ADB. A volume of 10 µl will be used per assay.
3. Prepare 10 µl aliquots of a 1mCi/100µl stock of [γ-^{32}]ATP (100µCi/vial). Before beginning the assay, dilute an aliquot to 1µCi/µl with 90µl 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 µg/µl PRAK Substrate Peptide.
3. Add 10 µl of diluted PRAK, active (200 ng purified enzyme per assay).
4. Add 10 µl of the diluted [γ-^{32}P]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

1. New, L. *et al*., PRAK, a novel protein kinase regulated by the p38 MAP Kinase. EMBO J., **17**, 3372-3384 (1998).
2. Raingeaud, J., *et al*., Pro-inflammatory cytokines and environmental stress cause p38 MAP kinase activation by dual phosphorylation on tyrosine and threonine. J. Biol. Chem., **270**, 7420-7426 (1995).
3. Foltz, I., *et al*., Hemopoietic growth factors with the exception of interleukin-4 activate the p38 mitogen activated protein kinase pathway. J. Biol. Chem., **272**, 3296-3301 (1997).

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