

Certificate of Analysis

Product	p38alpha peptide substrate, RRRLVEPLTPSGEAPNQK
Cat No	PKS-017-01
Lot No	0710060202
Description	The synthetic peptide RRRLVEPLTPSGEAPNQK can be used as a substrate for p38alpha kinase in <i>in vitro</i> kinase assays. Its sequence is derived from epidermal growth factor receptor (amino acids 643 - 656 of accession number AAH94761) M.W. 2048
Purity	95 % (by HPLC)
Form	Lyophilized powder Reconstitution of 1 mg in 245 µl H ₂ O dest. results in a 2 mM solution used in the p38alpha activity assay.
Package size	1 mg
Storage condition	-20 °C
Shipment conditions	room temperature

Material for in vitro research use only. Not for pharmaceutical or drug application. Material does not contain any animal products such as albumin.

AVOID FREEZE/THAW CYCLES

***In vitro* Kinase Assay**

Assay Components

Assay buffer (AB): 50 mM Tris-HCl, pH 7.5, 0.1 mM EGTA, 10 mM DTT

Enzyme dilution buffer: AB with 1 mg/ml BSA

Substrate: p38alpha peptide substrate RRRLVEPLTPSGEAPNQK, 2 mM

Protein kinase: p38alpha, 1.25 - 10 ng/ microliter diluted in EDB directly before use

Magnesium/ATP Cocktail: 80 mM MgCl₂, 4 microM ATP

Diluted [γ -³²P]ATP: Mix 197 microliter Magnesium/ATP cocktail with 3 microliter (30 microCi) [γ -³²P]ATP (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig)

Assay procedure

All compounds are pipetted into a microcentrifuge tube on ice

1. Add 10 microliter AB
2. Add 10 microliter peptide substrate
3. Add 2.5 microliter p38alpha (12.5 - 100 ng/assay)
4. Add 10 microliter of the diluted [γ -³²P]ATP
5. Incubate 10 min at 30 °C.
6. Stop the reaction by setting samples on ice
7. Remove 20 microliter and spot on P81 paper (let bind to the paper for 30 sec)
8. Immerse the paper in 0.75% phosphoric acid, gently shake on a rotator
9. Wash 3 x with phosphoric acid
10. Wash 1 x with acetone
11. Dry under infrared light
12. Read in scintillation counter or Instant Imager