KinaseDetect ApS
http://www.kinasedetect.com

Technical support:
Phone: +45 66 10 66 62
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Certificate of Analysis

Product	**CK2 peptide substrate 2, RRRADDSDDDDD**
Cat No	PKS-010-01
Lot No	PT1031050202 2
Description	The synthetic peptide RRRADDSDDDDD can be used as a substrate for protein kinase CK2alpha subunit or holoenzyme in *in vitro* kinase assays. It is phosphorylated by CK2 holoenzyme with a K_m of 20 micro and a Vmax of 232.1 nmol/min/mg. The peptide is highly specific for CK2, since at position -3, where an acidic residue is specifically required by CK1, but not by CK2, an Ala has been located. M.W. 1,450
Purity	90 - 95 % (by HPLC)
Form	Lyophilized powder Reconstitution of 1 mg in 1.725 ml H_2O dest. results in a 400 microM solution used on the CK2 activity assay.
Package size	1 mg
Storage condition	-20 ^{O}C
Shipment conditions	room temperature

References

Meggio F, Marin O, Pinna LA. (1994) Substrate specificity of protein kinase CK2. Cell Mol Biol Res 40, 401-9.

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

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In vitro Kinase Assay

Assay Components

One-for-all-buffer (OFAB): 20 mM Tris-HCl, 25 mM beta-glycerol phosphate, 5 mM EGTA, 1 mM DTT, pH 7.5
Substrate**:** CK2 peptide 2 RRRADDSDDDDD, 400 microM
Protein kinase**:** CK2alpha1 5 - 10 ng/microliter diluted in OFAB directly before use
Magnesium/ATP Cocktail**:** 75 mM $MgCl_2$, 500 microM ATP
Diluted [gamma–^{32}P]ATP: Mix 197 microliter Magnesium/ATP cocktail with 3 microliter (30 microCi) [gamma–^{32}P]ATP (3000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

Assay Procedure

All compounds are pipetted into a microcentrigue tube on ice
1. Add 10 microliter OFAB
2. Add 10 microliter 400 microM CK2 peptide 2
3. Add 10 microliter CK2alpha1 (50 - 100 ng/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 min at 37 ºC.
6. Stop the reaction by setting samples on ice
7. Remove 10 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