KinaseDetect ApS
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e-mail:support@kinasedetect.com

Certificate of Analysis

Product	**CK2 peptide substrate 1, RRRDDDSDDD**
Cat No	PKS-009-01
Lot No	918-140305
Description	The synthetic peptide RRRDDDSDDD 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 50 microM and a Vmax of 159.7 nmol/min/mg. M.W. 1,264
Purity	90 - 95 % (by HPLC)
Form	Lyophilized powder Reconstitution of 1 mg in 1 ml H_2O dest. results in a 800 microM solution used in the CK2 activity assay.
Package size	1 mg
Storage condition	-20 ^{O}C
Shipment conditions	room temperature

References

Kuenzel EA, Mulligan JA, Sommercorn J, Krebs EG (1987) Substrate specificity determinants for casein kinase II as deduced from studies with synthetic peptides. J Biol Chem 262, 9136-9140.
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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CK2alpha *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 RRRDDDSDDD, 800 microM

Protein kinase**:** CK2alpha 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 microl 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 800 microM CK2 peptide
3. Add 10 microliter CK2alpha (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