

Certificate of Analysis

Product	CHK peptide substrate, KKKVSRSGLYRSPSPENLNRPR
Cat No	PKS-007-01
Lot No	827-120704
Description	The synthetic peptide KKKVSRSGLYRSPSPENLNRPR can be used as a substrate for checkpoint kinases CHK1 and CHK2 in <i>in vitro</i> kinase assays. Its sequence is derived from cdc25C. M.W. 2,701
Purity	90 - 95 % (by HPLC)
Form	Lyophilized powder Reconstitution of 1 mg in 1.85 ml H ₂ O dest. results in a 200 microM solution used in the CHK2 activity assay.
Package size	1 mg
Storage condition	-20 °C
Shipment conditions	room temperature

References

O'Neill T, Giarratani L et al. (2002) Determination of substrate motifs for human Chk1 and hCds1/Chk2 by the oriented peptide library approach. *J Biol Chem* 277:16102-15.

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

CHK2 *in vitro* Kinase Assay

Assay components

Reaction buffer: 40 mM MOPS pH 7.5, 2 mM EDTA

Substrate: CHK peptide (KKKVSRSGLYRSPSPENLNRPR) , 200 microM

Protein kinase: CHK2, 100 ng/ 3 microliter diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 5 % glycerol, 10 mM DTT, 1 mg/ml BSA

Magnesium/ATP Cocktail: 37.5 mM MgCl₂, 250 microM ATP

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

Assay procedure

All compounds are pipetted into a microcentrifuge tube on ice

1. Add 4 microliter reaction buffer
2. Add 3 microliter 200 microM CHK peptide
3. Add 3 microliter CHK2 enzyme (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 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.