

Certificate of Analysis

Product	GSK3 peptide substrate, YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE
Cat No	PKS-012-01
Lot No	786-190304
Description	The synthetic peptide YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE can be used as a substrate for GSK3alpha and beta in <i>in vitro</i> kinase assays. M.W. 3,028
Purity	90 - 95 % (by HPLC)
Form	Lyophilized powder Reconstitution of 1 mg in 1.3 ml H ₂ O dest. results in a 250 micromM solution used in the GSK3beta activity assay.
Package size	1 mg
Storage condition	-20 °C
Shipment conditions	room temperature

References

Sutherland C, Leighton IA, Cohen P (1993) Inactivation of glycogen synthase kinase-3 beta by phosphorylation: kinase connection in insulin and growth factor signalling. *Biochem J* 296 (Pt 1):15-9.

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

GSK3beta *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 sodium orthovanadate, 1 mM DTT, pH 7.5

Substrate: GSK3 peptide (YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE), 250 microM

Protein kinase: GSK3beta 5 mU/microliter, diluted in OFAB directly before use

Magnesium/ATP Cocktail: 75 mM MgCl₂, 500 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, Germany)

Assay Procedure

All compounds are pipetted into a microcentrifuge tube on ice

1. Add 10 microliter OFAB
2. Add 10 microliter GSK3 peptide, YRRAAVPPSPSLSRHSSPHQ(pS)EDEEE, 250 microM
3. Add 10 microliter GSK3beta (50 mU/assay)
4. Add 10 microliter of the diluted [γ -³²P]ATP
5. Incubate 30 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