KinaseDetect ApS
http://www.kinasedetect.com

Technical support:
Phone: +45 66 10 66 62
e-mail:support@kinasedetect.com

Certificate of Analysis

Product	**AKT/PKB peptide substrate, RPRAATF**
Cat No	PKS-003-01
Lot No	503-280502
Description	The synthetic peptide RPRAATF can be used as a substrate for AKT/PKB in *in vitro* kinase assays. It is phosphorylated by AKT1/PKBalpha with a K_m of 25 microM. M.W. 818
Purity	85 - 90 % (by HPLC)
Form	Lyophilized powder Reconstitution in 3 ml H_2O dest. results in a 400 microM solution used in the AKT1/PKBalpha activity assay.
Package size	1 mg
Storage condition	-20 ^{O}C
Shipment conditions	room temperature

References

Alessi DR, Caudwell FB, Andjelkovic M, Hemmings BA, Cohen P (1996) Molecular basis for the substrate specificity of protein kinase B: comparison with MAPKAP kinase-1 and p70 S6 kinase. FEBS Lett 399:333-8.

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

KinaseDetect ApS
http://www.kinasedetect.com

Technical support:
Phone: +45 66 10 66 62
e-mail:support@kinasedetect.com

AKT1/PKBalpha *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: AKT peptide, RPRAATF, 400microM
Protein kinase: AKT1, 20 ng/microliter diluted in OFAB
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 (3,000 Ci/mmol, e.g. from Hartmann Analytic, Braunschweig, Germany)

Assay procedure
All compounds are pipetted into a microcentrigue tube on ice
1. Add 10 miroliter OFAB
2. Add 10 microliter 400 microM AKT peptide
3. Add 10 microliter Akt1enzyme (200 ng/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]ATP
5. Incubate 10 min at 30 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