KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product	**PIM1 peptide substrate, KKRNRTLTV**
Cat.No.	PKS-019-01
Lot No	081210
Description	The synthetic peptide KKRNRTLTV can be used as a substrate for PIM1 in *in vitro* kinase assays. M.W. 1.135,14
Purity	90 - 95 % (by HPLC)
Form	Lyophilized powder Reconstitution of 1 mg in 2.2 ml H_2O dest. results in a 400 microM solution used in the PIM1 activity assay.
Package size	1 mg
Storage condition	-20 ^{O}C
Shipment conditions	room temperature

References

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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PIM-1 *In vitro* Kinase Assay

Assay components

Assay buffer (AB): 50 mM Tris-HCl, 10 mM DTT, pH 7.5
Enzyme dilution buffer (EB): Assay buffer containing 0,1 % CHAPS
Substrate: PIM-1 peptide, KKRNRTLTV, 400 microM in H2O
Protein kinase: PIM-1, 1 - 5 ng/microliter diluted in EB
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 microliter AB
2. Add 10 microliter 400 microM PIM-1 peptide
3. Add 10 microliter PIM-1 (10 - 50 ng/assay)
4. Add 10 microliter of the diluted [gamma–^{32}P]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