KinaseDetect ApS
http://www.kinasedetect.com

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

Certificate of Analysis

Product	**PTP1B, catalytic domain**
Cat No	PP-001-01
Lot No	1410110
Description	Purified recombinant human protein tyrosine phosphatase, non-receptor type 1 (PTP1B), catalytic domain, expressed in E.coli. N-terminally tagged with GST. Purified by affinity chromatography. M.W. 64.3 KDa Approved HUGO gene symbol: PTPN1

Quality

Protein concentration (Bradford with BSA as standard)	0.2 mg/ml
Purity	> 90 % by SDS PAGE
Activity	Diluting PTP1B 1/3200 in assay buffer will give a rate of 25 Fluorescence Units/minute at pH 7.0, room temperature, using 50uM DIFMUP as substrate. Diluting PTP1B 1/200 in assay buffer will give a rate of 0.03 OD/min at pH 7.0, room temperature, using 5mM pNPP as substrate.

Form	Liquid. In 25mM Hepes, 0.8mM Tris, 50% glycerol, 6mM DTT, 0.17mM glutathione, 0.08mM EDTA, pH 8.0
Package size	5 microgram
Storage condition	-80 ^{O}C
Shipment conditions	dry ice

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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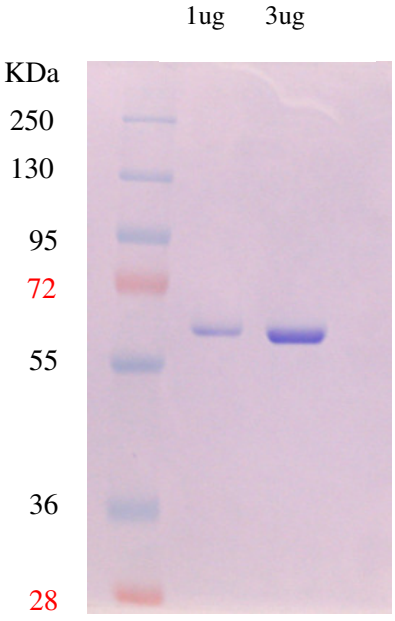
Amino acid sequence information

GST-PTP1B sequence: Factor-Xa in red and PTP1B (amino acid 1-321, NM_002927) in bold

MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL 50
EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL 100
DIRYGVSRIA YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH 150
PDFMLYDALD VVLYMDPMCL DAFPKLVCFK KRIEAIPQID KYLKSSKYIA 200
WPLQGWQATF GGGDHPPKSD **IEGR**GIP**MEM EKEFEQIDKS GSWAAIYQDI 250**
RHEASDFPCR VAKLPKNKNR NRYRDVSPFD HSRIKLHQED NDYINASLIK 300
MEEAQRSYIL TQGPLPNTCG HFWEMVWEQK SRGVVMLNRV MEKGSLKCAQ 350
YWPQKEEKEM IFEDTNLKLT LISEDIKSYY TVRQLELENL TTQETREILH 400
FHYTTWPDFG VPESPASFLN FLFKVRESGS LSPEHGPVVV HCSAGIGRSG 450
TFCLADTCLL LMDKRKDPSS VDIKKVLLEM RKFRMGLIQT ADQLRFSYLA 500
VIEGAKFIMG DSSVQDQWKE LSHEDLEPPP EHIPPPPRPP KRILEPHN 548

SDS-PAGE analysis



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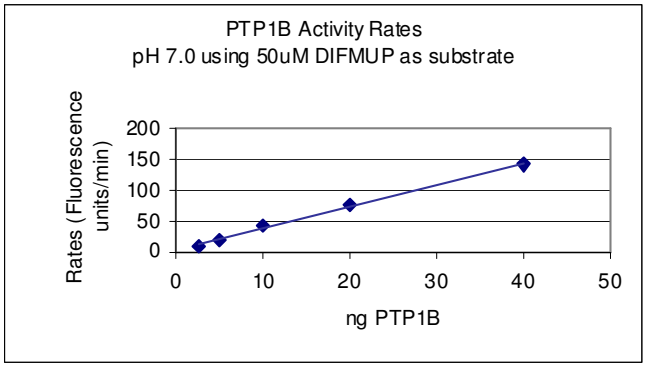
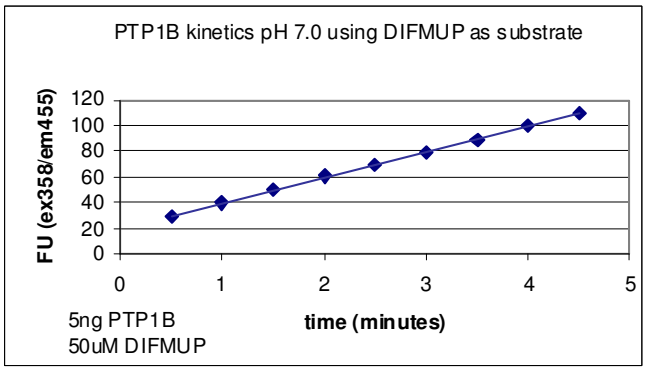
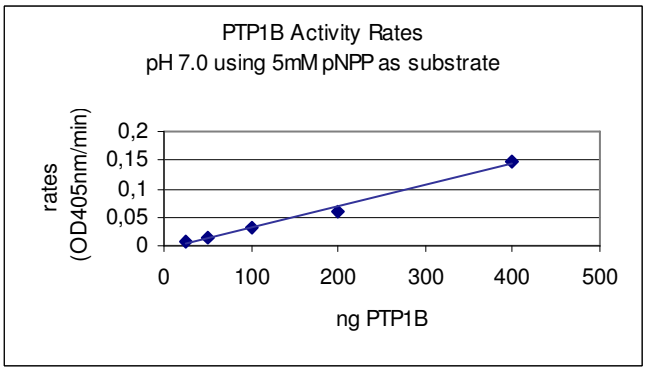
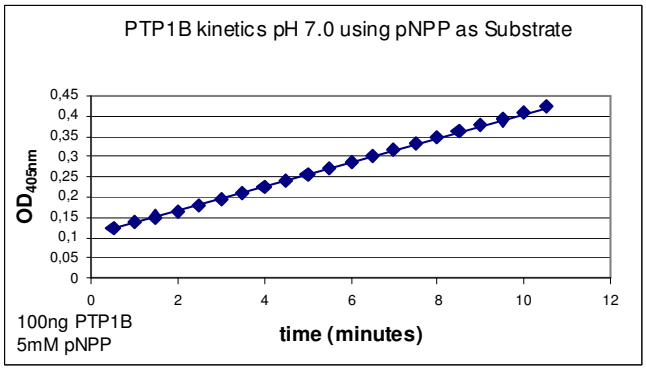
Activity









In vitro Phosphatase assay in microplate using DIFMUP as substrate

Material:

Assay buffer (AB) : 50mM Hepes, pH7.0, BSA 0.1mg/mL(add fresh) and DTT 3mM (add fresh)
Substrate: DIFMUP, 50uM final
GST-PTP1B: final dilution 1/3200 in assay buffer
Black 96 well plate, flat bottom
Plate reader able to measure kinetic fluorescence: Excitation 358nm/emission 455nm

Assay procedure:

1.Prepare AB, make enough for 100uL per sample: add DTT + BSA fresh
2.Prepare 2X enzyme dilution in AB: will need 50uL per well
3.Prepare 2X pNPP dilution: will need 50uL per well
4.Plate 50uL enzyme dilution per well in the 96 well plate
5.Add 50uL pNPP dilution per well
6. Incubate 1 minute at room temperature
7: Monitor fluorescence in plate reader at Excitation 358nm/emission 455nm at 30sec. intervals for 10-15 minutes, room temperature.

In vitro Phosphatase assay in microplate using pNPP as substrate

Material:

Assay buffer (AB): 50mM Hepes, pH7.0, BSA 0.1mg/mL (add fresh) and DTT 3mM (add fresh)
Substrate: pNPP, 5mM final
GST-PT1BP: final dilution 1/200 in assay buffer
Clear 96 well plate, flat bottom
Plate reader able to measure kinetic absorbance at 405nM

Assay procedure:

Same assay procedure as for DIFMUP but step 7. Monitor absorbance in plate reader at 405nM at 30sec. intervals for 10-15 minutes, room temperature.