*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

## Preparation of active TcPINK1 [1 – 570] D359A

**Enzyme description:-** TcPINK1 [1 – 570] D359A

**Clone number:-** DU 34832

**Source:-** Recombinant

**Expression system:-** *E.coli*

**Tag:-** N-terminal Maltose Binding Protein (MBP)

**Purification method**:- Amylose agarose

**Calculated molecular mass:-**
Monoisotopic 108, 085.51 daltons
Average Mass 108, 153.54 daltons
[cysteines reduced, methionines have not been oxidised

**Theoretical pI:-** 6.13

**Purity:-** 85 %

**Activation protocol:-** Constitutively active

**Enzyme storage buffer:-**
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

**Storage temperature:-** –70 °C

**Assay buffer:-**
50 mM Tris-HCl pH 7.5, 0.1 % 2–mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

**Substrate:-**
GST-PARK2 (1 – 108) [DU 37370]

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# Clone Data Sheet

## TcPINK1 [1 – 570] D359A

**Protein** TcPINK1 [1 - 570] D359A

**Clone number** DU 34832

**Species** *Tribolium castaneum*

**Accession number** XM_963274

**Tags** N-terminal MBP

**Bacterially expressed protein**

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLE
EKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKL
YPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPA
LDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIK
DVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAM
TINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAA
SPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELVKD
PRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDE
ALKDAQTNSSSNNNNNNNNNLGDDDDKVPEFLEVLFQGPGS**MSV
RAVGSRLFKHGRSLIQQFCKRDLNTTIGDKINAVSQATAAPSSLP
KTQIPKNFALRNVGVQLGLQARRILIDNVLNRVTNSLSAELRKKA
TRRILFGDSAPFFALVGVSIASGTGILTKEEELEGVCWEIREAIS
KIKWQYYDIDESRFESNPITLNDLSLGKPIAKGTNGVVYSAKVKD
DETDDNKYPFALKMMFNYDIQSNSMEILKAMYRETVPARMYYSNH
DLNNWEIELANRRKHLPPHPNIVAIFSVFTDLIQELEGSKDLYPA
ALPPRLHPEGEGRNMSLFLLMKRYDCNLQSFLSTAPSTRTSLLLL
AQLLEGVAHMTAHGIAHRDLKSDNLLLDTSEPESPILVIS**A**FGCC
LADKTNGLSLPYTSYEMDKGGNTALMAPEIICQKPGTFSVLNYSK
ADLWAVGAIAYEIFNCHNPFYGPSRLKNFNYKEGDLPKLPDEVPT
VIQALVANLLKRNPNKRLDPEVAANVCQLFLWAPSTWLKPGLKVP
TSGEILQWLLSLTTKVLCEGKINNKSFGEKFTRNWRRTYPEYLLI
SSFLCRAKLANVRNALHWIQENLPELD

**Native sequence** Amino acids M1 – D570 (end) of *Tribolium castaneum* PINK1. Residue M403 of the fusion protein is equivalent to M1 of the native enzyme. The MBP tag is located at residues 1 - 392.

The enzyme has a D359A mutation, which produces a kinase dead enzyme. Residue D359 is equivalent to A761 of the fusion protein.

**Protease cleavage** PreScission (LEVLFQGP) residues 392 - 400

**Cloning sites** *Bam*H1 and *Not*I sites of pMal (modified)