

Certificate of Analysis

PAR-1B α , active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-544, 14-544-K, 14-544M

Parent Lot # D8MN019U

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged, recombinant, full-length, human PAR-1B α , expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA. Purity 31.6% by SDS-PAGE and Coomassie blue staining. MW = 81.6kDa.

Specific Activity (Parent lot# D8MN019U): 1670U/mg, where one unit of PAR-1B- α , active activity is defined as 1nmol phosphate incorporated into 100 μ M CHKtide per minute at 30°C with a final ATP concentration of 100 μ M.

Formulation: 1.5215mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

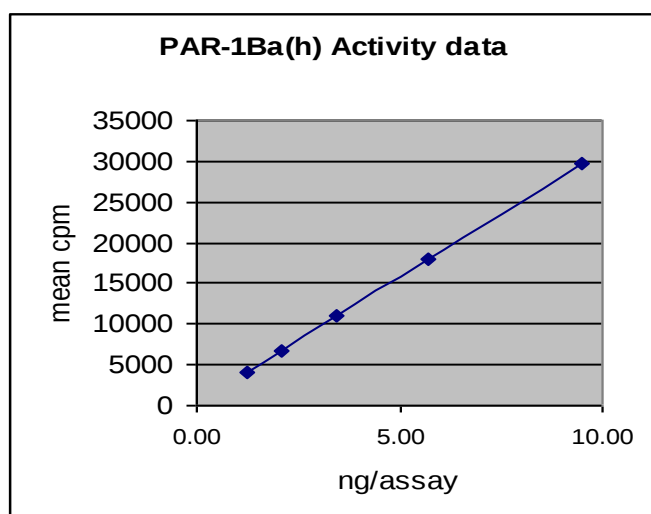
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C..

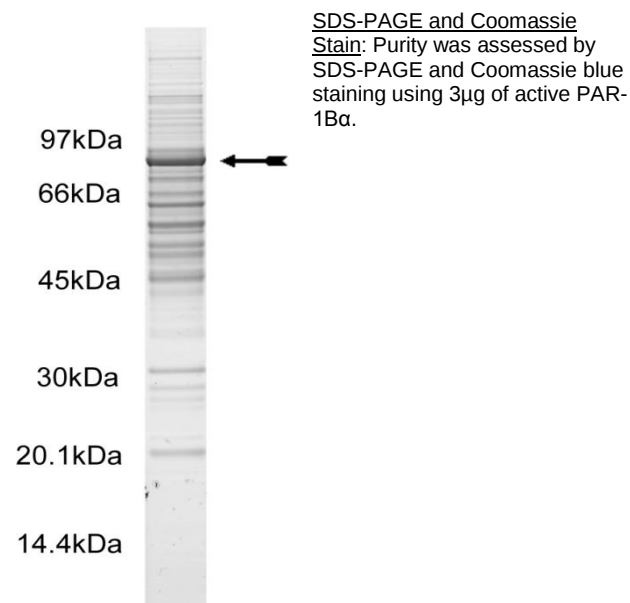
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 1.3–9.5ng of this lot of enzyme phosphorylated 100 μ M CHKtide in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity PAR-1B- α with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH 7.0, 1mM EDTA.
2. **CHKtide (KKKVSRSGLYRSPSPENLNRPR):** Use at a final assay concentration of 100 μ M. Prepare a 1mM stock and add 2.5 μ l of stock per assay point.
3. **PAR-1B-alpha, active:** Dilute with 20mM MOPS/NaOH pH 7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 1.3–9.5ng per assay point.
4. **[γ -³³P]ATP:** 2.5 x magnesium acetate/[γ -³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ -³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5 μ l of 5 x reaction buffer to wells.
2. Add 2.5 μ l of **CHKtide**.
3. Add **2.5 μ l (1.3–9.5ng) PAR-1B-alpha, active**.
4. Add 5 μ l of dH₂O.
5. Add 10 μ l of diluted [γ -³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5 μ l 3% phosphoric acid.
8. Transfer a 10 μ l aliquot onto the appropriate area of a **P30 Filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1 μ l of 30% phosphoric acid.

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PAR-1B α Sequence Information

<u>Protein</u>	Human PAR-1B α
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M33 of the recombinant protein is equivalent to M1 of human PAR-1B alpha
<u>Accession number</u>	GenBank AF387638

Recombinant PAR-1B α amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGMIRGRNSA TSADEQPHIG NYRLLKTIGK
61 GNFAKVKLAR HILTGKEVAV KIIDKTQLNS SSLQKLFREV RIMKVLNHPN IVKLFETIET
121 EKTLYLVMEY ASGGEVFDYL VAHGRMKEKE ARAKFRQIVS AVQYCHQKFI VHRDLKAENL
181 LLDADMNIKI ADFGFSNEFT FGNKLDTFEG SPPYAAPELF QGKKYDGPEV DVWSLGVILY
241 TLVSGSLPFD GQNLKELRER VLRGKYRIPF YMSTDCENLL KKFLILNPSK RGTLEQIMKD
301 RWMNVGHEDD ELKPYVEPLP DYKDPRTTEL MVSMGYTREE IQDSL VGQRY NEVMATYLLL
361 GYKSSELEGD TITLKPRPSA DLTNSSAPSP SHKVQRSVSA NPKQRRFSDQ AGPAIPTSNS
421 YSKKTQSNN AENKRPEEDRE SGRKASSTAK VPASPLPGL RKKTTPTPST NSVLSTSTNR
481 SRNSPLLEA SLGQASIQNG KDSTAPQRPV VASPSAHNIS SSGGAPDRTN FPRGVSSRST
541 FHAGQLRQVR DQQLNPYGV T PASPSGHSQG RRGASGSIFS KFTSKFVRRN LNEPESKDRV
601 ETLRPHVVG SGGNDKEKEEF REAKPRSLRF TWSMKTSSM EPNEMMREIR KVL DANCQS
661 ELHEKYMLLC MHGTPGHEDF VQWEMEVC KL PRLSLNGVRF KRISGTSMAF KNIASKIANE
721 LKL

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Recombinant PAR-1B α nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgccatgga tccggaattc aaaggtatga ttcggggccc caactcagcc
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721 aacttggtca gcggatccct gccttttgat ggacagaacc tcaaggagct gcgggaacgg
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1621 ttccatgctg ggcagctccg acaggtgcgg gaccagcaga atttgcctta cgggtgtgacc
1681 ccagcctctc cctctggcca cagccagggc cggcgggggg cctctgggag catcttcagc

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2101 aagcggatat cgggcacctc catggccttc aaaaacattg cctccaaaat agccaacgag
2161 ctgaagcttt aa

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