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Certificate of Analysis

TGFBR1, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-912, 14-912-K, 14-912M
Parent Lot # D10EP004N

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 GST-tagged, recombinant, human TGFBR1 amino acids 200–end containing a T204D mutation, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. The T204D mutation has been demonstrated to enhance kinase activity *in vitro* (Wieser *et al*, EMBO (1995);**14**:2199-2208).
Purity 98% by SDS-PAGE and Coomassie blue staining. MW = 62kDa.

Specific Activity (Parent lot# D10EP004N): 273U/mg, where one unit of TGFBR1 activity is defined as 1nmol phosphate incorporated into 2mg/ml casein per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.04mg/ml of enzyme in 50mM Tris/HCl pH7.0, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

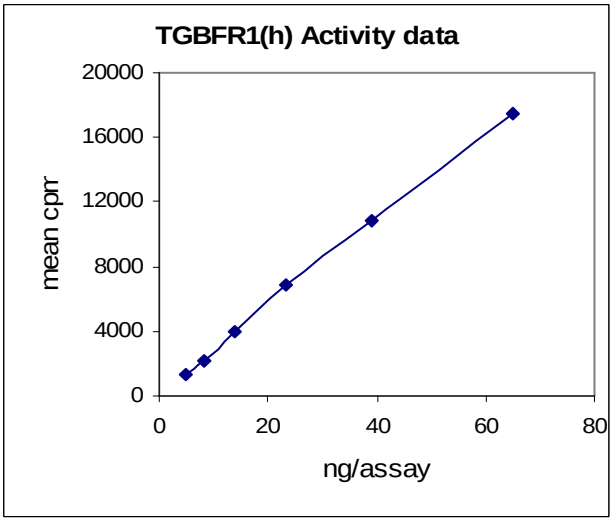
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

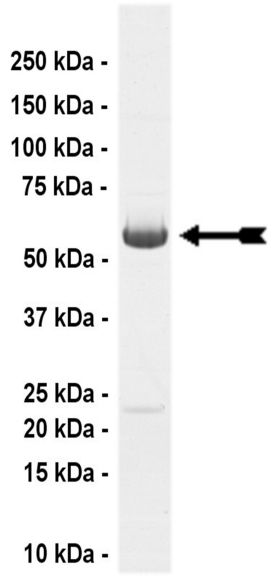
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 5–65ng of this lot of enzyme phosphorylated 2mg/ml casein 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 as TGFBR1 with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active TGFBR1.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA.
2. **Manganese Chloride:** Use at a final concentration of 1mM. Prepare a 100mM stock and use 0.25µl per well.
3. **TGFBR1, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 5–65ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x MgAc/[γ^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ^{33}P]ATP (specific activity approximately 500 - 800cpm/pmol as required).

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer per assay to wells.
2. Add 0.25 µl of 100mM manganese chloride
3. Add 4.75µl of dH_2O.
4. Add 2.5µl of 20mg/ml casein.
5. Add **2.5µl (5–65ng) TGFBR1, active.**
6. Add 10µl of diluted [γ-^{33}P]ATP mixture.
7. Incubate for 10 minutes at 30°C.
8. Stop the reaction by adding 5µl of 3% phosphoric acid.
9. Transfer a 10µl aliquot onto the appropriate area of a **P30 Filtermat.**
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with methanol.
12. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
13. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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TGFBR1 Sequence Information

Protein human TGFBR1

Tags *N*-terminal GST

Native sequence T237 of the recombinant protein is equivalent to T200 of human TGFBR1

Accession number GenBank NM_004612

Recombinant TGFBR1 amino acid sequence:

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   1 MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
  61 GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
 121 DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
 181 KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPEF KGLRRQTIAR
 241 DIVLQESIGK GRFGEVWRGK WRGEEVAVKI FSSREERSWF REAEIYQTVM LRHENILGFI
 301 AADNKDNGTW TQLWLVSDYH EHGSLFDYLN RYTVTVEGMI KLALSTASGL AHLHMEIVGT
 361 QGKPAIAHRD LKSKNILVKK NGTCCIADLG LAVRHDSATD TIDIAPNHRV GTKRYMAPEV
 421 LDDSINMKHF ESFKRADIYA MGLVFWEIAR RCSIGGIHED YQLPYYDLVP SDPSVEEMRK
 481 VVCEQKLRPN IPNRWQSCEA LRVMAKIMRE CWYANGAARL TALRIKKTLS QLSQQEGIKM
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Recombinant TGFBR1 nucleotide sequence:

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   1 atgtccccta tactaggtta ttggaaaatt aagggccttg tgcaacccac tcgacttctt
  61 ttggaatatc ttgaagaaaa atatgaagag catttgtatg agcgcgatga aggtgataaa
 121 tggcgaaaca aaaagtttga attgggtttg gagtttccca atcttcctta ttatattgat
 181 ggtgatgtta aattaacaca gtctatggcc atcatacgtt atatagctga caagcacaac
 241 atgttgggtg gttgtccaaa agagcgtgca gagatttcaa tgcttgaagg agcggttttg
 301 gatattagat acggtgtttc gagaattgca tatagtaaag actttgaaac tctcaaagtt
 361 gattttctta gcaagctacc tgaaatgctg aaaatgttcg aagatcgttt atgtcataaa
 421 acatatttaa atggtgatca tgtaacccat cctgacttca tgttgtatga cgctcttgat
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 541 aaacgtattg aagctatccc acaaattgat aagtacttga aatccagcaa gtatatagca
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 961 gagcatggat ccctttttga ttacttaaac agatacacag ttactgtgga aggaatgata
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1081 caaggaaagc cagccattgc tcatagagat ttgaaatcaa agaatatctt ggtaaagaag
1141 aatggaactt gctgtattgc agacttagga ctggcagtaa gacatgattc agccacagat
1201 accattgata ttgctccaaa ccacagagtg ggaacaaaaa ggtacatggc ccctgaagtt
1261 ctcgatgatt ccataaatat gaaacatttt gaatccttca aacgtgctga catctatgca
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1441 gttgtttgtg aacagaagtt aaggccaaat atcccaaaca gatggcagag ctgtgaagcc
1501 ttgagagtaa tggctaaaat tatgagagaa tgttggtatg ccaatggagc agctaggctt
1561 acagcattgc ggattaagaa aacattatcg caactcagtc aacaggaagg catcaaaatg
1621 taa
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Reviewed and approved by site quality representative.