

Certificate of Analysis

TGFBFR1, 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 TGFBFR1 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 TGFBFR1, active 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.

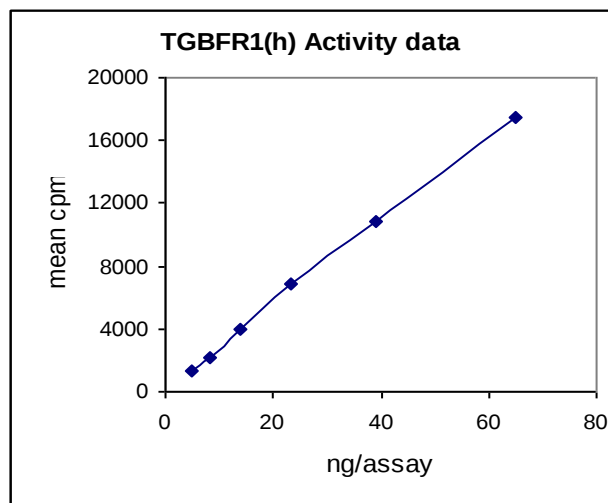
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

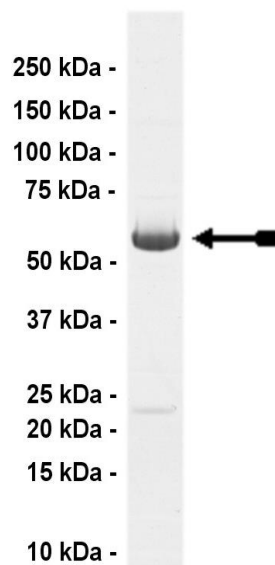
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 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 TGFBFR1 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 TGFBFR1, active.

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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-³³P]ATP:** 2.5 x MgAc/[γ³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ³³P]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 dH₂O.
4. Add 2.5µl of 20mg/ml casein.
5. Add **2.5µl (5–65ng) TGFBR1, active.**
6. Add 10µl of diluted [γ-³³P]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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TGFR1 Sequence Information

Protein	human TGFR1
Tags	N-terminal GST
Native sequence	T237 of the recombinant protein is equivalent to T200 of human TGFR1
Accession number	GenBank NM_004612

Recombinant TGFR1 amino acid sequence:

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1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQ SMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFLSKLPEML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAIPOID  KYLKSSKYIA  WPLQGWQATF  GGDHPPKSD  LEVLFQGPEF  KGLRRQTIAR
241  DIVLQESIGK  GRFGEVWRGK  WRGEEVAVKI  FSSREERSWF  REAEIYQTVM  LRHENILGFI
301  AADNKDNGTW  TQLWLVS DYH  EHGS LFDYLN  RYTVTVEGMI  KLALSTASGL  AHLHMEIVGT
361  QGKPAIAHRD  LKSKNILVKK  NGTCCIADLG  LAVRHDSATD  TIDIAPNHRV  GTKRYMAPEV
421  LDDSINMKHF  ESFKRADIYA  MGLVFWEIAR  RCSIGGIHED  YQLPYYDLVP  SDPSVEEMRK
481  VVCEQKL RPN  IPNRWQSC EA  LRVMAKIMRE  CWYANGAARL  TALRIKKTLS  QLSQQEGIKM

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Recombinant TGFR1 nucleotide sequence:

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1  atgtccccta  tactagggtta  ttggaaaatt  aagggccttg  tgcaaccac  tcgacttctt
61  ttggaatatc  ttgaagaaaa  atatgaagag  catttgtatg  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatatgtat
181  ggtgatgtta  aattaacaca  gtctatggcc  atcatacggt  atatagtga  caagcacaa
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
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1501  ttgagagtaa  tggctaaaat  tatgagagaa  tggttggtat  ccaatggagc  agctaggctt
1561  acagcattgc  ggattaagaa  aacattatcg  caactcagtc  aacaggaagg  catcaaaatg
1621  taa

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