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Certificate of Analysis

Tie2, active

(Recombinant enzyme expressed in Sf21 insect cells)

Catalogue # 14-540

Lot # 1611488

Product Description: N-terminal 6His-tagged recombinant human Tie2 residues 771–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 76% by SDS-PAGE and Coomassie blue staining. MW = 42kDa.

Specific Activity (lot# 1611488): 58U/mg, where one unit of Tie2 activity is defined as 1nmol phosphate incorporated into 0.1 mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 10µg of enzyme in 3.9µl of 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.

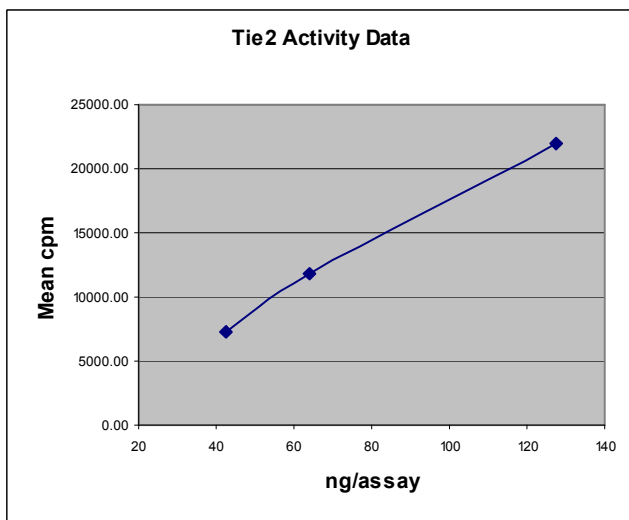
Storage and Stability: Store at -70°C from date of shipment. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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**

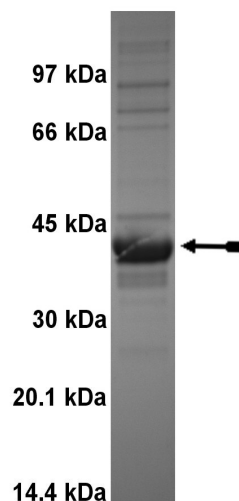
Quality Control Testing

Kinase Assay: 42–127ng of this lot of enzyme phosphorylated 0.1 mg/ml poly(Glu, Tyr) (4:1) 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 Tie2 with 51% amino acid coverage of 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 Tie2, active.



Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA
2. **Manganese Chloride (MnCl₂):** Use at a final assay concentration of 0.5mM. Prepare a 10mM stock and add 1.25µl of stock per assay point.
3. **Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock and add 2.5µl of stock per assay point.
4. **Tie2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 42–127ng per assay point.
5. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.2mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 5µl of 5 x reaction buffer to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add 1.25µl of MnCl₂.
4. Add **2.5µl (42–127ng) Tie2, active**.
5. Add 3.75µl of dH₂O.
6. Add 10 µl 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 **Filtermat A**.
10. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
11. Wash the filtermat once for 2 minutes with acetone.
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.

Tie2 Sequence Information

Protein	human Tie2
Tags	N-terminal 6His
Native sequence	Q10 of the recombinant protein is equivalent to Q771 of human Tie2
Accession number	GenBank NM_000459. The recombinant protein contains the amino acid substitutions Q939H and Q940H (native protein coordinates) with respect to GenBank NM_000459; both of these are reported in GenBank BC035514.

Recombinant Tie2 amino acid sequence:

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1 MHHHHHHEFQ LKRANVQRRM AQAFQNVREE PAVQFNSGTL ALNRKVKNNP DPTIYPVLDW
61 NDIKFQDVIG EGNFGQVLKA RIKKDGLRMD AAIKRMKEYA SKDDHRDFAG ELEVLCKLGH
121 HPNIINLLGA CEHRGYLYLA IEYAPHGNLL DFLRKSRLVE TDPAFAIANS TASTLSSHHL
181 LHFAADVARG MDYLSQKQFI HRDLAARNIL VGENYVAKIA DFGLSRGQEV YVKKTMGRLP
241 VRWMAIESLN YSVYTTNSDV WSYGVLLWEI VSLGGTPYCG MTCAELYEKL PQGYRLEKPL
301 NCDDEVYDLM RQCWREKPYE RPSFAQILVS LNRMLEERKT YVNTTLYEKF TYAGIDCSAE
361 EAA
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Recombinant Tie2 nucleotide sequence:

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1 atgcatcatc accatcacca tgaattccaa ttgaagaggg caaatgtgca aaggagaatg
61 gcccaagcct tccaaaacgt gaggaagaa ccagctgtgc agttcaactc agggactctg
121 gccctaaaca ggaaggtcaa aaacaacca gatcctacaa tttatccagt gcttgactgg
181 aatgacatca aatttcaaga tgtgattggg gagggcaatt ttggccaagt tcttaaggcg
241 cgcacaaaga aggatgggtt acggatggat gctgccatca aaagaatgaa agaatatgcc
301 tccaaagatg atcacaggga ctttgacagga gaactggaag ttctttgtaa acttggacac
361 catccaaaca tcatcaatct cttaggagca tgtgaacatc gaggctactt gtacctggcc
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481 acggaccagc catttgccat tgccaatagc accgcgtcca cactgtcctc ccatcatctc
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601 cacagggatc tggctgccag aacatttta gttggtgaaa actatgtggc aaaaatagca
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841 atgacttgtg cagaactcta cgagaagctg ccccagggct acagactgga gaagcccctg
901 aactgtgatg atgaggtgta tgatctaatag agacaatgct ggccgggagaa gccttatgag
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1021 tacgtgaata ccacgcttta tgagaagttt acttatgcag gaattgactg ttctgctgaa
1081 gaagcggcct ag
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