

Certificate of Analysis

Tie2 (Y897S), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-764, 14-764-K, 14-764M

Parent Lot # D7JN033U

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 human Tie2 residues 771–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

The Y897S substitution is a gain-of-function mutation associated with hereditary venous malformations (Calvert et al., (1999), Hum. Mol. Genet. **8**, 1279-1289).

Purity 93% by SDS-PAGE and Coomassie blue staining. MW = 42kDa.

Formulation: 2.9mg/ml of enzyme in 50mM Tris/HCl pH8.5, 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.

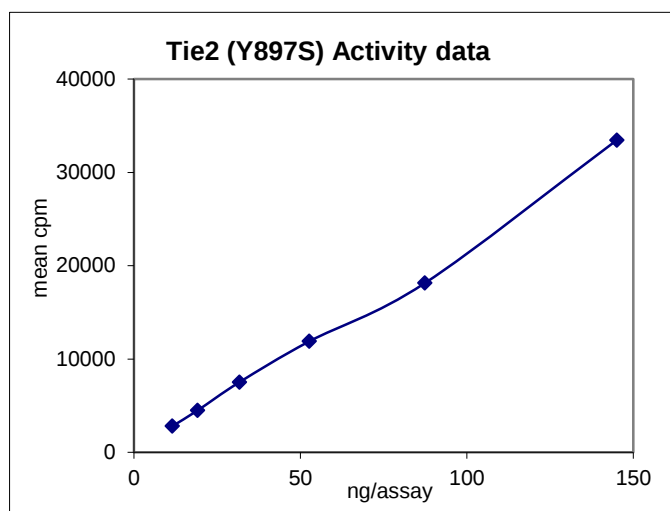
Specific Activity (Parent lot# D7JN033U): As provided, this lot demonstrated 123U/mg, where one unit of Tie2 (Y897S), active activity is defined as 1nmol phosphate incorporated into 400µM (EFPIYDFLPK) per minute at 30°C with a final ATP concentration of 100µM.

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**

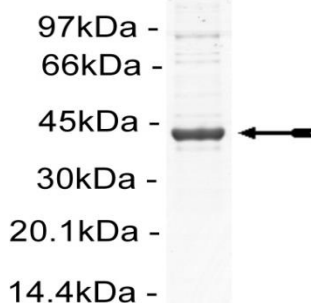
Quality Control Testing

Kinase Assay: 12–145ng of this lot of enzyme phosphorylated 400µM (EFPIYDFLPK) 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 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 (Y897S), 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 (MnCl₂):** Use at a final assay concentration of 2.5mM. Prepare a 100mM stock and add 0.625µl of stock per assay point.
3. **(EFPIYDFLPAKKK):** Use at a final assay concentration of 400µM. Prepare a 4mM stock and add 2.5µl of stock per assay point.
4. **Tie2 (Y897S) active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 12–145ng per assay point.
5. **[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 5µl of 5 x reaction buffer to wells.
2. Add 2.5µl of **(EFPIYDFLPAKKK)**.
3. Add 0.625µl of MnCl₂.
4. Add **2.5µl (12–145ng) Tie2 (Y897S), active**.
5. Add 4.375µl of dH₂O.
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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Tie2 Sequence Information

<u>Protein</u>	human Tie2 (771–end, Y897S)
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	Q10 of the recombinant protein is equivalent to Q771 of human Tie2
<u>Accession number</u>	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 (Y897S) amino acid sequence:

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

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Recombinant Tie2 (Y897S) nucleotide sequence:

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1  atgcatcatc accatcacca tgaattccaa ttgaagaggg caaatgtgca aaggagaatg
61  gcccaagcct tccaaaacgt gagggagaag ccagctgtgc agttcaactc agggactctg
121 gccctaaaca ggaaggtcaa aaacaaccca gatcctacaa tttatccagt gcttgactgg
181 aatgacatca aatttcaaga tgtgattggg gagggcaatt ttggccaagt tcttaaggcg
241 cgcacaaaga aggatgggtt acggatggat gctgccatca aaagaatgaa agaatatgcc
301 tccaaagatg atcacaggga ctttgaggga gaactggaag ttctttgtaa acttggaacac
361 catccaaaca tcacatcatc cttaggagga tgtgaacatc gaggtcctt gtacctggcc
421 attgagtacg cgcccatgg aaaccttctg gacttccttc gcaagagccg tgtgctggag
481 acggacccag catttgccat tgccaatagc accgcgtcca cactgtcctc ccatcatctc
541 cttcacttcg ctgccgacgt ggcccggggc atggactact tgagccaaaa acagtttatc
601 cacagggatc tggctgccag aaacatttta gttggtgaaa actatgtggc aaaaatagca
661 gattttggat tgtcccgagg tcaagagggt tatgtgaaaa agacaatggg aaggctccca
721 gtgcgctgga tggccatcga gtcactgaat tacagtgtgt acacaaccaa cagtgatgta
781 tggtcctatg gtgtgttact atgggagatt gttagcttag gaggcacacc ctactcgagg
841 atgacttgtg cagaactcta cgagaagctg cccagggct acagactgga gaagcccctg
901 aactgtgatg atgaggtgta tgatctaata agacaatgct ggcgggagaa gccttatgag
961 aggccatcat ttgccagat attggtgtcc ttaaacagaa tgttagagga gcgaaagacc
1021 tacgtgaata ccacgcttta tgagaagttt acttatgcag gaattgactg ttctgctgaa
1081 gaagcgcct ag

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