

Certificate of Analysis

Tie2 (R849W), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-763, 14-763-K, 14-763M

Parent Lot # 1611523

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 containing the R849W substitution, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose.

The R849W mutation has been reported in dominantly inherited venous malformations. Expression studies in insect and mammalian cells demonstrate that Tie2 R849W has constitutively elevated tyrosine kinase activity compared with wild type. (Morris P.N., *et al.*, Biochem.Biophys.Res.Commun.(2006);**346**:335-338 and Vikkula M, *et al.*, Cell(1996);**87**:1181-1190).

Purity 88.1% by SDS-PAGE and Coomassie blue staining. MW = 42kDa.

Formulation: 1.916mg/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.

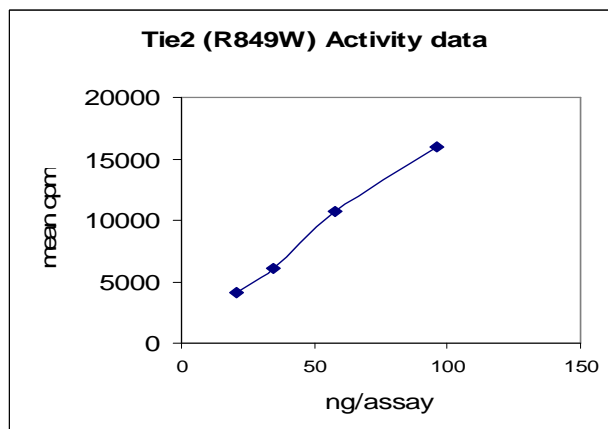
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

Specific Activity (Parent lot# 1611523): As provided, this lot demonstrated 196U/mg, where one unit of Tie2 (R849W) activity is defined as 1nmol phosphate incorporated into 400µM (EFPIYDFLPAK) per minute at 30°C with a final ATP concentration of 100µM.

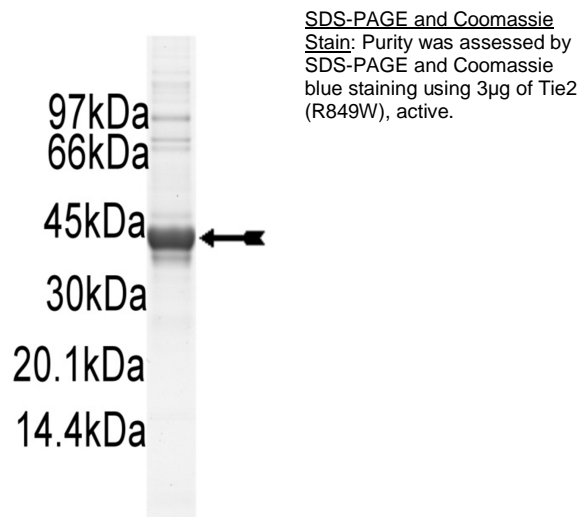
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 20.9–95.8ng of this lot of enzyme phosphorylated 400µM (EFPIYDFLPAK) 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.



Certificate of Analysis

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 (R849W), active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 20.9–95.8ng 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 (20.9–95.8ng) Tie2 (R849W), 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.

Certificate of Analysis

Tie2 Sequence Information

<u>Protein</u>	human Tie2 (771–end, R849W)
<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 (R849W) amino acid sequence:

```

1  MHHHHHHEFQ  LKRANVQRRM  AQAFQNVREE  PAVQFNSGTL  ALNRKVKNNP  DPTIYPVLDW
61  NDIKFQDVIG  EGNFGQVLKA  RIKKDGLWMD  AAIKRMKEYA  SKDDHRDFAG  ELEVLCKLGH
121 HPNIIINLLGA CEHRGYLYLA  IEYAPHGNLL  DFLRKSRLVE  TDPAFAIANS  TASTLSSHLL
181 LHFAADVARG  MDYLSQKQFI  HRDLAARNIL  VGENYVAKIA  DFGLSRGQEV  YVKKTMGRPL
241 VRWMAIESLN  YSVYTTNSDV  WSYGVLLWEI  VSLGGTPYCG  MTCAELYEKL  PQGYRLEKPL
301 NCDDEVYDLM  RQCWREKPYE  RPSFAQILVS  LNRMLEERKT  YVNTTLYEKF  TYAGIDCSAE
361  EAA

```

Recombinant Tie2 (R849W) nucleotide sequence:

```

1  atgcatcatc  accatcacca  tgaattccaa  ttgaagaggg  caaatgtgca  aaggagaatg
61  gcccaagcct  tccaaaacgt  gagggaagaa  ccagctgtgc  agttcaactc  agggactctg
121 gccctaaaca  ggaaggtcaa  aaacaaccca  gatcctacaa  tttatccagt  gcttgactgg
181 aatgacatca  aatttcaaga  tgtgattggg  gagggcaatt  ttggccaagt  tottaaggcg
241 cgcacatca  aggatgggtt  atggatggat  gctgccatca  aaagaatgaa  agaatatgcc
301 tccaaagatg  atcacaggga  ctttgcagga  gaactggaag  ttctttgtaa  acttggacac
361 catccaaaca  tcatcaatct  cttaggagca  tgtgaacatc  gaggctactt  gtacctggcc
421 attgagtagc  cgccccatgg  aaaccttctg  gacttccttc  gcaagagccg  tgtgctggag
481 acggaccocag  catttgccat  tgccaatagc  accgcgtcca  cactgtcctc  ccatcatctc
541 cttcacttcg  ctgcccagct  ggccccgggg  atggactact  tgagccaaaa  acagtttatc
601 cacagggatc  tggctgccag  aaacatttta  gttggtgaaa  actatgtggc  aaaaatagca
661 gattttggat  tgtcccagg  tcaagaggtg  tatgtgaaaa  agacaatggg  aaggctccca
721 gtgcgctgga  tggccatcga  gtcactgaat  tacagtgtgt  acacaacca  cagtgatgta
781 tggtcctatg  gtgtgttact  atgggagatt  gttagcttag  gaggcacacc  ctactgctgg
841 atgacttgtg  cagaactcta  cgagaagctg  ccccagggct  acagactgga  gaagcccctg
901 aactgtgatg  atgaggtgta  tgatctaata  agacaatgct  ggccgggaga  gccttatgag
961 aggccatcat  ttgccagat  attggtgtcc  ttaaacagaa  tgtagtagga  gcgaaagacc
1021 tacgtgaata  ccacgcttta  tgagaagttt  acttatgcag  gaattgactg  ttctgctgaa
1081 gaagcggcct  ag

```

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.