eurofins | Discovery Services

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 Ni^{2+}/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.

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 (EFPIYDFLPAKKK) per minute at 30°C with a final ATP concentration of 100µM.

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.

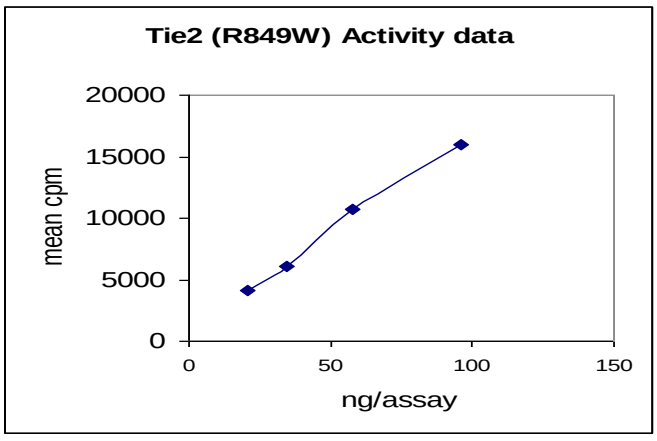
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

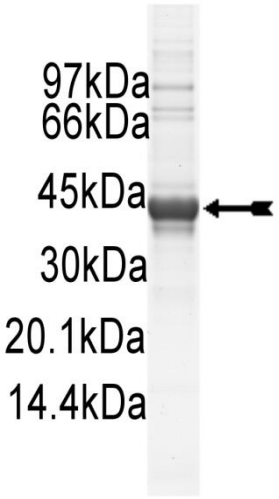
FOR *IN VITRO* RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 20.9–95.8ng of this lot of enzyme phosphorylated 400µM (EFPIYDFLPAKKK) 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 (R849W), active.

Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom
T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

eurofins | Discovery Services

Certificate of Analysis

Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA
2. **Manganese Chloride ($MnCl_2$):** 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-^{33}P]ATP:** 2.5 x magnesium acetate/[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 to wells.
2. Add 2.5µl of **(EFPIYDFLPAKKK)**.
3. Add 0.625µl of $MnCl_2$.
4. Add **2.5µl (20.9–95.8ng) Tie2 (R849W), active**.
5. Add 4.375µl of dH_2O.
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.

eurofins | Discovery Services

Certificate of Analysis

Tie2 Sequence Information

Protein human Tie2 (771–end, R849W)

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 (R849W) amino acid sequence:

```
  1 MHHHHHHEFQ LKRANVQRRM AQAFQNVREE PAVQFNSGTL ALNRKVKNNP DPTIYPVLDW
 61 NDIKFQDVIG EGNFGQVLKA RIKKDGLWMD AAIKRMKEYA SKDDHRDFAG ELEVLCKLGH
121 HPNIINLLGA CEHRGYLYLA IEYAPHGNLL DFLRKSRVLE 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
```

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 tcttaaggcg
 241 cgcatcaaga aggatgggtt atggatggat gctgccatca aaagaatgaa agaatatgcc
 301 tccaaagatg atcacaggga ctttgcagga gaactggaag ttctttgtaa acttggacac
 361 catccaaaca tcatcaatct cttaggagca tgtgaacatc gaggctactt gtacctggcc
 421 attgagtacg cgccccatgg 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 tcaagaggtg tatgtgaaaa agacaatggg aaggctccca
 721 gtgcgctgga tggccatcga gtcactgaat tacagtgtgt acacaaccaa cagtgatgta
 781 tggtcctatg gtgtgttact atgggagatt gttagcttag gaggcacacc ctactgcggg
 841 atgacttgtg cagaactcta cgagaagctg ccccagggct acagactgga gaagcccctg
 901 aactgtgatg atgaggtgta tgatctaatg agacaatgct ggcgggagaa gccttatgag
 961 aggccatcat ttgcccagat attggtgtcc ttaaacagaa tgttagagga gcgaaagacc
1021 tacgtgaata ccacgcttta tgagaagttt acttatgcag gaattgactg ttctgctgaa
1081 gaagcggcct ag
```

Reviewed and approved by site quality representative.