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Certificate of Analysis

ErB4, active

(Recombinant enzyme expressed in Sf21 insect cells)
Item # 14-569, 14-569-K, 14-569M
Parent Lot # 25635U

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 ErB4 amino acids 706-991, expressed by baculovirus in Sf21 insect cells. Purified using Ni^{2+}/NTA-agarose. Purity 81% by SDS-PAGE and Coomassie blue staining. MW = 36.1kDa.

Specific Activity (Parent lot# 25635U): 451U/mg, where one unit of ErB4, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 2.65mg/ml of enzyme in 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.

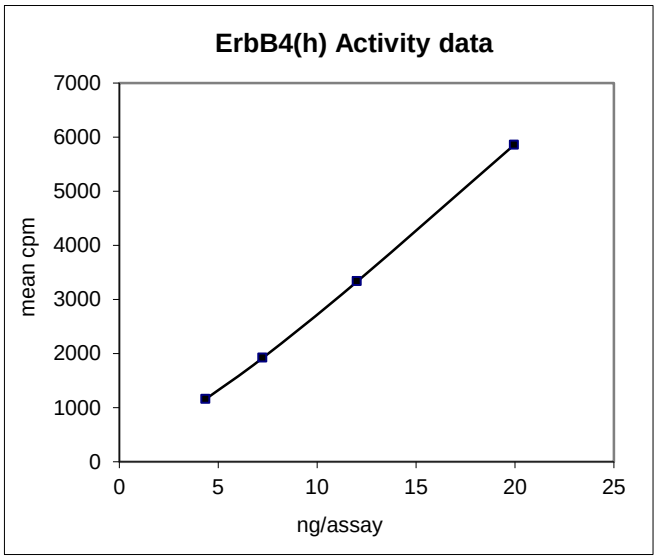
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

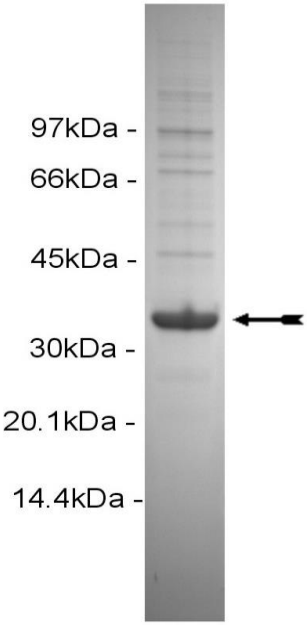
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 4–20ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) 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 ErB4, active with the translated native sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of ErB4, 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 ($MnCl_2$):** Use at a final assay concentration of 2.5mM. Prepare a 50mM 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. **ErB4, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 5% glycerol, 0.01% Brij-35, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4–20ng 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 per assay to appropriate wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (4–20ng) ErB4, active**.
4. Add 1.25µl 50mM $MnCl_2$
5. Add 3.75µl of dH_2O.
6. Add 10µl of diluted [γ-^{33}P]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 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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ErbB4 Sequence Information

Protein Human ErbB4

Tags *N*-Terminal 6His

Native sequence N29 of the recombinant protein is equivalent to N706 of human ErbB4

Accession number GenBank NM_005235

Recombinant ErbB4 amino acid sequence:

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  1 MSYYHHHHHH DYDIPTTENL YFQGAMGSNQ AQLRILKETE LKRVKVLGSG AFGTVYKGIW
 61 VPEGETVKIP VAIKILNETT GPKANVEFMD EALIMASMDH PHLVRLLGVC LSPTIQLVTQ
121 LMPHGCLLEY VHEHKDNIGS QLLLNWCVQI AKGMMYLEER RLVHRDLAAR NVLVKSPNHV
181 KITDFGLARL LEGDEKEYNA DGGKMPIKWM ALECIHYRKF THQSDVWSYG VTIWELMTFG
241 GKPYDGIPTR EIPDLLEKGE RLPQPPICTI DVYMVMVKCW MIDADSRPKF KELAAEFSRM
301 ARDPQRYLVI QGDD
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Recombinant ErbB4 nucleotide sequence:

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  1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
 61 tattttcagg gcgccatggg atccaatcaa gctcaacttc gtattttgaa agaaactgag
121 ctgaagaggg taaaagtcct tggctcaggt gcttttggaa cggtttataa aggtatttgg
181 gtacctgaag gagaaactgt gaagattcct gtggctatta agattcttaa tgagacaact
241 ggtcccaagg caaatgtgga gttcatggat gaagctctga tcatggcaag tatggatcat
301 ccacacctag tccggttgct gggtgtgtgt ctgagcccaa ccatccagct ggttactcaa
361 cttatgcccc atggctgcct gttggagtat gtccacgagc acaaggataa cattggatca
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Reviewed and approved by site quality representative.