

Discovery Services

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 6Histagged, recombinant, human ErB4 amino acids 706-991, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTAagarose. Purity 81% by SDS-PAGE and Coomassie blue staining. MW = 36.1kDa.

Specific Activity (Parent lot# 25635U): 451U/mg, where one unit of ErB4 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 6 months 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.

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

Quality Control Testing

<u>Kinase Assay</u>: 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.



<u>MS Tryptic Fingerprint:</u> Confirmed identity as ErB4, active with the translated native sequence listed on page three.



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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 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.
- 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. $[\gamma^{-33}P]ATP$: 2.5 x magnesium acetate/ $[\gamma^{-33}P]ATP$ cocktail: 25mM MgAc and 0.25mM ATP to which is added $[\gamma^{-33}P]ATP$ (specific activity approximately 500 800cpm/pmol as required.)

Assay Procedure (96 well plate format):

- 1. Add 5μ I 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 MnCl₂
- 5. Add 3.75 μ l of dH₂O.
- 6. Add 10µl of diluted $[\gamma^{-33}P]$ 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:

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
421	caactgctgc	ttaactggtg	tgtccagata	gctaagggaa	tgatgtacct	ggaagaaaga
481	cgactcgttc	atcgggattt	ggcagcccgt	aatgtcttag	tgaaatctcc	aaaccatgtg
541	aaaatcacag	attttgggct	agccagactc	ttggaaggag	atgaaaaaga	gtacaatgct
601	gatggaggaa	agatgccaat	taaatggatg	gctctggagt	gtatacatta	caggaaattc
661	acccatcaga	gtgacgtttg	gagctatgga	gttactatat	gggaactgat	gacctttgga
721	ggaaaaccct	atgatggaat	tccaacgcga	gaaatccctg	atttattaga	gaaaggagaa
781	cgtttgcctc	agcctcccat	ctgcactatt	gacgtttaca	tggtcatggt	caaatgttgg
841	atgattgatg	ctgacagtag	acctaaattt	aaggaactgg	ctgctgagtt	ttcaaggatg
901	gctcgagacc	ctcaaagata	cctagttatt	cagggtgatg	attag	

Reviewed and approved by site quality representative.

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