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Certificate of Analysis

ErbB2, active
(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-939, 14-939-K, 14-939M

Parent Lot # D13NP005N

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: *C*-terminal 6His-tagged, recombinant, human ErbB2 amino acids 676-end expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.
Purity 85% by SDS-PAGE and Coomassie blue staining. MW = 69kDa.

Specific Activity (Parent lot# D13NP005N): 361U/mg, where one unit of ErbB2 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: 1.871mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.5mM EDTA, 0.01% CHAPS, 50% glycerol, 2mM DTT. Frozen solution.

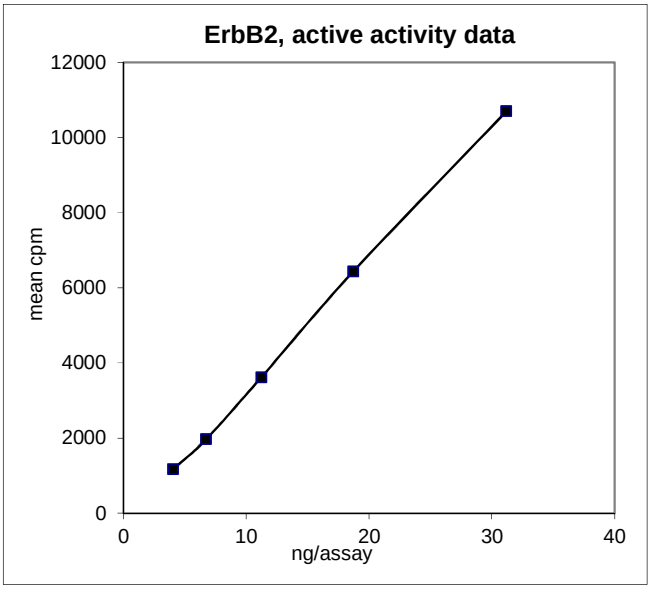
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

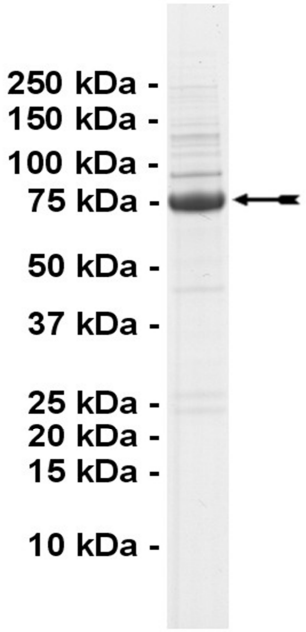
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 4–32ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) 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 ErbB2 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 ErbB2, active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 40mM MOPS/NaOH pH7.0, 1mM EDTA
2. **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.
3. **Manganese Chloride**: Use at a final assay concentration of 5mM. Prepare a 100mM stock and add 1.25µL per well.
4. **ErbB2, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4-32ng per assay point.
5. **[γ-^{33}P]ATP:** 2.5 x MgAc/[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 wells.
2. Add 2.5µl of **poly(Glu,Tyr) (4:1).**
3. Add 1.25µL 100mM manganese chloride.
4. Add 3.75µl of dH_2O.
5. Add **2.5µl (4–32ng) ErbB2, active**.
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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ErbB2 Sequence Information

Protein human ErbB2

Tags *C*-terminal 6His

Native sequence K19 and V598 of the recombinant protein are equivalent to K676 and V1255 of human ErbB2 respectively.

Accession number GenBank NM_004448
The recombinant protein contains the amino acid substitution G778D with reference to GenBank NM_004448. The G778D mutation has been shown to promote folding of the recombinant protein into an active conformation (Fan *et al*, J. Biol. Chem. **283**, 1588-1596, 2008).

Recombinant ErbB2 amino acid sequence:

```
   1 MLVPRGSPWI RNSKAYVDKR RQQKIRKYTM RRLLQETELV EPLTPSGAMP NQAQMRILKE
  61 TELRKVKVLG SGAFGTVYKG IWIPDGENVK IPVAIKVLRE NTSPKANKEI LDEAYVMAGV
 121 DSPYVSRLLG ICLTSTVQLV TQLMPYGCLL DHVRENRGRL GSQDLLNWCM QIAKGMSYLE
 181 DVRLVHRDLA ARNVLVKSPN HVKITDFGLA RLLDIDETEY HADGGKVPIK WMALESILRR
 241 RFTHQSDVWS YGVTVWELMT FGAKPYDGIP AREIPDLLEK GERLPQPPIC TIDVYMIMVK
 301 CWMIDSECRP RFRELVSEFS RMARDPQRFV VIQNEDLGPA SPLDSTFYRS LLEDDDMGDL
 361 VDAEEYLVPQ QGFFCPDPAP GAGGMVHHRH RSSSTRSGGG DLTLGLEPSE EEAPRSPLAP
 421 SEGAGSDVFD GDLGMGAAKG LQSLPTHDPS PLQRYSEDPT VPLPSETDGY VAPLTCSPQP
 481 EYVNQPDVRP QPPSPREGPL PAARPAGATL ERPKTLSPGK NGVVKDVFAF GGAVENPEYL
 541 TPQGGAAPQP HPPPAFSPAF DNLYYWDQDP PERGAPPSTF KGTPTAENPE YLGLDVPVCS
 601 RHASGTDDYD IATTHHHHHH
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Recombinant ErbB2 nucleotide sequence:

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   1 atgttggtcc cgcgtggctc gccgtggatc cgtaactcga aagcctacgt cgacaagcga
  61 cggcagcaga agatccggaa gtacacgatg cggagactgc tgcaggaaac ggagctggtg
 121 gagccgctga cacctagcgg agcgatgccc aaccaggcgc agatgcggat cctgaaagag
 181 acggagctga ggaaggtgaa ggtgcttgga tctggcgctt ttggcacagt ctacaagggc
 241 atctggatcc ctgatgggga gaatgtgaaa attccagtgg ccatcaaagt gttgagggaa
 301 aacacatccc ccaaagccaa caaagaaatc ttagacgaag catacgtgat ggctggtgtg
 361 gactccccat atgtctcccg ccttctgggc atctgcctga catccacggt gcagctggtg
 421 acacagctta tgccctatgg ctgcctctta gaccatgtcc gggaaaaccg cggacgcctg
 481 ggctcccagg acctgctgaa ctggtgtatg cagattgcca aggggatgag ctacctggag
 541 gatgtgcggc tcgtacacag ggacttggcc gctcggaacg tgctggtcaa gagtcccaac
 601 catgtcaaaa ttacagactt cgggctggct cggctgctgg acattgacga gacagagtac
 661 catgcagatg ggggcaaggt gcccatcaag tggatggcgc tggagtccat tctccgccgg
 721 cggttcaccc accagagtga tgtgtggagt tatggtgtga ctgtgtggga gctgatgact
 781 tttggggcca aaccttacga tgggatccca gcccgggaga tccctgacct gctggaaaag
 841 ggggagcggc tgccccagcc ccccatctgc accattgatg tctacatgat catggtcaaa
 901 tgttggatga ttgactctga atgtcggcca agattccggg agttggtgtc tgaattctcc
 961 cgcatggcca gggacccccа gcgctttgtg gtcatccaga atgaggactt gggcccagcc
1021 agtcccttgg acagcacctt ctaccgctca ctgctggagg acgatgacat gggggacctg
1081 gtggatgctg aggagtatct ggtaccccag cagggcttct tctgtccaga ccctgccccg
1141 ggcgctgggg gcatggtcca ccacaggcac cgcagctcat ctaccaggag tggcggtggg
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1261 tccgaagggg ctggctccga tgtatttgat ggtgacctgg gaatgggggc agccaagggg
1321 ctgcaaagcc tccccacaca tgaccccagc cctctacagc ggtacagtga ggaccccaca
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1381 gtacccctgc cctctgagac tgatggctac gttgcccccc tgacctgcag cccccagcct
1441 gaatatgtga accagccaga tgttcggccc cagccccctt cgccccgaga gggccctctg
1501 cctgctgccc gacctgctgg tgccactctg gaaaggccca agactctctc cccagggaag
1561 aatggggtcg tcaaagacgt ttttgccttt gggggtgccg tggagaaccc cgagtacttg
1621 acaccccagg gaggagctgc ccctcagccc caccctcctc ctgccttcag cccagccttc
1681 gacaacctct attactggga ccaggaccca ccagagcggg gggctccacc cagcaccttc
1741 aaagggacac ctacggcaga gaacccagag tacctgggtc tggacgtgcc agtgtgttcg
1801 cgtcacgcct cgggcacgga cgattacgac attgccacca cgcatcacca tcaccatcac
1861 taa
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Reviewed and approved by site quality representative.