Certificate of Analysis

REACTION BIOLOGY

Recombinant Proteins

ProQinase™ ERBB2 wt

erb-b2 receptor tyrosine kinase 2

Recombinant Human Active Protein Kinase

HGNC Symbol: ERBB2

Synonyms: HER-2, NEU, NGL, TKR1, CD340

Product No.: 0108-0000-1

Lot: 015

Description: Human ERBB2 wt, C-terminal fragment, amino acids Q_{679}-V_{1255} (as in NCBI/Protein entry NP_004439.2), N-terminal GST-HIS_6 fusion protein with a Thrombin cleavage site, expressed in Sf9 insect cells

Product identity: ERBB2 wt Lot 015, was confirmed as ERBB2 wt by mass spectroscopy LC-ESI-MS/MS

Theoretical $MW_{Fusion\ Protein}$: 93,908 Da

Expression host: Sf9 insect cells

Purification: GST-Affinity Chromatography

Activation: This kinase was not activated by special procedures

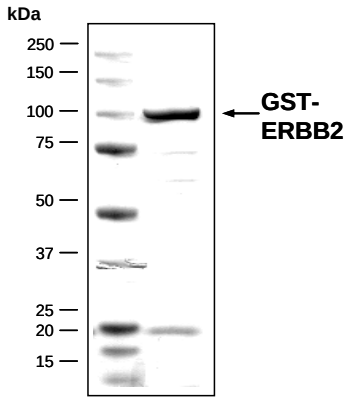
Storage buffer: 50 mM HEPES pH 7.5, 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione, 20 % glycerol

Storage temperature: -80°C
For complete recovery, mix well and spin before use. Product must not be stored in diluted solutions, aliquots below 10µl are not advisable. Avoid repeated freeze-thaw cycles!

Protein concentration: 0.224 µg/µl
(Bradford method using BSA [Sigma, cat# A-7638, Lot 79H7641] as standard protein)

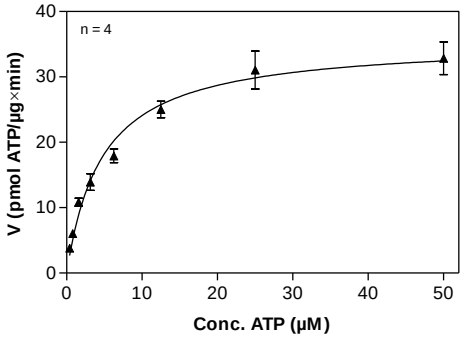
Biochemical Parameters:
Specific kinase activity (P_i transfer): 36 pmol/µg × min
ATP-K_M: 4.8 µM

ERBB2 wt Lot 015:
Coomassie stain



2.0 µg GST-ERBB2

ERBB2 wt Lot 015:
Determination of V_{max} and K_M value for ATP



Determination of K_M value & Specific activity:
- Assay conditions:
  60 mM HEPES-NaOH, pH 7.5
  3 mM $MgCl_2$
  3 mM $MnCl_2$
  3 µM Na-orthovanadate
  1.2 mM DTT
  50 µg/ml $PEG_{20.000}$
  ATP (variable)
  Substrate: $Poly(Glu:Tyr)_{4:1}$, 5 µg/ml
  Kinase: 4 µg/ml
- Filter binding assay
  MSFC membrane (Millipore)

Additional assay technology:
ERBB2 wt Lot 015 was also successfully tested by Reaction Biology for the use with the ADP-Glo™ Kinase assay from Promega ADP-Glo assay conditions may vary from radiometric assay conditions, please inquire for assay details

ProQinase™ ERBB2

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GST-ERBB2 Recombinant Fusion Protein Amino Acid Sequence

1	MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID	60
61	GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV	120
121	DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK	180
181	KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD PMGHHHHHHG RRRASVAAGI	240
241	LVPRGSPGLD GICSIEEFRP PWQQKIRKYT MRRLLQETEL VEPLTPSGAM PNQAQMRILK	300
301	ETELRKVKVL GSGAFGTVYK GIWIPDGENV KIPVAIKVLR ENTSPKANKE ILDEAYVMAG	360
361	VGSPYVSRLL GICLTSTVQL VTQLMPYGCL LDHVRENRGR LGSQDLLNWC MQIAKGMSYL	420
421	EDVRLVHRDL AARNVLVKSP NHVKITDFGL ARLLDIDETE YHADGGKVPI KWMALESILR	480
481	RRFTHQSDVW SYGVTVWELM TFGAKPYDGI PAREIPDLLE KGERLPQPPI CTIDVYMIMV	540
541	KCWMIDSECR PRFRELVSEF SRMARDPQRF VVIQNEDLGP ASPLDSTFYR SLLEDDDMGD	600
601	LVDAEEYLVP QQGFFCPDPA PGAGGMVHHR HRSSSTRSGG GDLTLGLEPS EEEAPRSPLA	660
661	PSEGAGSDVF DGDLGMGAAK GLQSLPTHDP SPLQRYSEDP TVPLPSETDG YVAPLTCSPQ	720
721	PEYVNQPDVR PQPPSPREGP LPAARPAGAT LERPKTLSPG KNGVVKDVFA FGGAVENPEY	780
781	LTPQGGAAPQ PHPPPAFSPA FDNLYYWDQD PPERGAPPST FKGTPTAENP EYLGLDVPV	840

1-218: GST **Red**: HIS6-tag **Pink**: Thrombin cleavage site **blue**: ERBB2 fragment

ERBB2 wt[1] Amino Acid Sequence

1	MELAALCRWG LLLALLPPGA ASTQVCTGTD MKLRLPASPE THLDMLRHLY QGCQVVQGNL	60
61	ELTYLPTNAS LSFLQDIQEV QGYVLIAHNQ VRQVPLQRLR IVRGTQLFED NYALAVLDNG	120
121	DPLNNTTPVT GASPGGLREL QLRSLTEILK GGVLIQRNPQ LCYQDTILWK DIFHKNNQLA	180
181	LTLIDTNRSR ACHPCSPMCK GSRCWGESSE DCQSLTRTVC AGGCARCKGP LPTDCCHEQC	240
241	AAGCTGPKHS DCLACLHFNH SGICELHCPA LVTYNTDTFE SMPNPEGRYT FGASCVTACP	300
301	YNYLSTDVGS CTLVCPLHNQ EVTAEDGTQR CEKCSKPCAR VCYGLGMEHL REVRAVTSAN	360
361	IQEFAGCKKI FGSLAFLPES FDGDPASNTA PLQPEQLQVF ETLEEITGYL YISAWPDSLP	420
421	DLSVFQNLQV IRGRILHNGA YSLTLQGLGI SWLGLRSLRE LGSGLALIHH NTHLCFVHTV	480
481	PWDQLFRNPH QALLHTANRP EDECVGEGLA CHQLCARGHC WGPGPTQCVN CSQFLRGQEC	540
541	VEECRVLQGL PREYVNARHC LPCHPECQPQ NGSVTCFGPE ADQCVACAHY KDPPFCVARC	600
601	PSGVKPDLSY MPIWKFPDEE GACQPCPINC THSCVDLDDK GCPAEQRASP LTSIISAVVG	660
661	ILLVVVLGVV FGILIKRRQQ KIRKYTMRRL LQETELVEPL TPSGAMPNQA QMRILKETEL	720
721	RKVKVLGSGA FGTVYKGIWI PDGENVKIPV AIKVLRENTS PKANKEILDE AYVMAGVGSP	780
781	YVSRLLGICL TSTVQLVTQL MPYGCLLDHV RENRGRLGSQ DLLNWCMQIA KGMSYLEDVR	840
841	LVHRDLAARN VLVKSPNHVK ITDFGLARLL DIDETEYHAD GGKVPIKWMA LESILRRRFT	900
901	HQSDVWSYGV TVWELMTFGA KPYDGIPARE IPDLLEKGER LPQPPICTID VYMIMVKCWM	960
961	IDSECRPRFR ELVSEFSRMA RDPQRFVVIQ NEDLGPASPL DSTFYRSLLE DDDMGDLVDA	1020
1021	EEYLVPQQGF FCPDPAPGAG GMVHHRHRSS STRSGGGDLT LGLEPSEEEA PRSPLAPSEG	1080
1081	AGSDVFDGDL GMGAAKGLQS LPTHDPSPLQ RYSEDPTVPL PSETDGYVAP LTCSPQPEYV	1140
1141	NQPDVRPQPP SPREGPLPAA RPAGATLERP KTLSPGKNGV VKDVFAFGGA VENPEYLTPQ	1200
1201	GGAAPQPHPP PAFSPAFDNL YYWDQDPPER GAPPSTFKGT PTAENPEYLG LDVPV	1260

blue: ERBB2 sequence expressed in recombinant protein

[1]NCBI/Protein accession number NP_004439.2

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