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Certificate of Analysis

mTOR (1362–end), active

(Recombinant enzyme expressed in Sf21 insect cells)
Item #14-770, 14-770-K, 14-770M
Parent Lot # WAD0182

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 FLAG™-tagged, recombinant, human mTOR, amino acids 1362–end, expressed by baculovirus in Sf21 insect cells. Purified using FLAG™ agarose. Purity 55% by SDS-PAGE and Coomassie blue staining. MW =137.3kDa.

Specific Activity (Parent lot# WAD0182): 186U/mg, where one unit of mTOR, active activity is defined as 1nmole of phosphate incorporated into 2mg/ml mTOR substrate (cat#. 12-645-K) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.29mg/ml of enzyme in 10mM HEPES, 50mM NaCl, 50mM β-glycerophosphate, 0.25mM sodium orthovanadate, 10mM NaF, 300µg/ml FLAG™ peptide, 20% v/v glycerol.

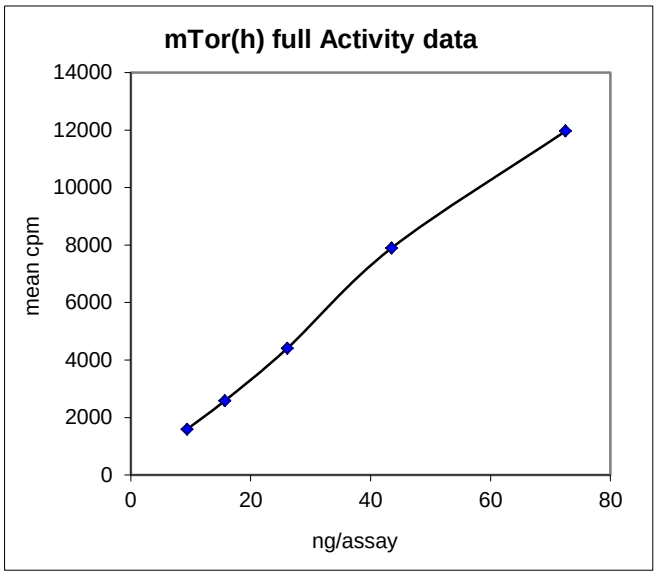
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

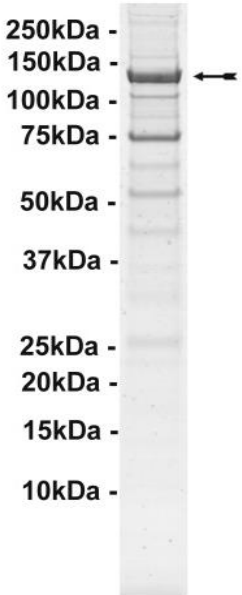
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase assay: 9.4–72.5ng of this lot of enzyme phosphorylated 2mg/ml mTOR substrate (cat#. 12-645-K) 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 product identity as mTOR (1362–end) 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 mTOR (1362–end), active.

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mTOR Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 450mM HEPES pH7.5, 9mM EGTA, 0.09% Tween-20.
2. **10 x Dilution Buffer:** 500mM HEPES pH7.5, 10mM EGTA, 0.1% Tween-20.
3. **mTOR substrate:** The substrate (cat#. 12-645-K) is used at a final assay concentration of 2mg/ml.
4. **mTOR (1362–end), active:** Dilute with 10 x dilution buffer. Use 2.5µl (9.4–72.5ng) per assay point.
5. **[γ-^{33}P]ATP:** 5 x manganese chloride/[γ-^{33}P]ATP cocktail: 15mM $MnCl_2$ and 0.5mM ATP to which is added [γ-^{33}P]ATP (specific activity approximately 250 - 400cpm/pmol as required.)

Assay Procedure (96 well plate format):

1. Add 2.5µl of 10 x reaction buffer per assay to wells.
2. Add **mTOR substrate** to a concentration of 2mg/ml (50µg per assay point in a 25µl reaction).
3. Add **2.5µl (9.4–72.5ng) mTOR (1362–end), active**.
4. Make up to 20µl with dH_2O.
5. Add 5µl of [γ-^{33}P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **P30 filtermat**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. 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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mTOR Sequence Information

Protein	Human mTOR
Tags	*N*-terminal FLAG™
Native sequence	E10 of recombinant sequence is equivalent to E1362 of human mTOR
Accession number	GenBank NM_004958

Recombinant mTOR amino acid sequence:

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   1 MDYKDDDDKE FMEHSDKGPL PLRDDNGIVL LGERAAKCRA YAKALHYKEL EFQKGPTPAI
  61 LESLISINNK LQQPEAAAGV LEYAMKHFGE LEIQATWYEK LHEWEDALVA YDKKMDTNKD
 121 DPELMLGRMR CLEALGEWGQ LHQQCCEKWT LVNDETQAKM ARMAAAAAWG LGQWDSMEEY
 181 TCMIPRDTHD GAFYRAVLAL HQDLFSLAQQ CIDKARDLLD AELTAMAGES YSRAYGAMVS
 241 CHMLSELEEV IQYKLVPERR EIIRQIWWER LQGCQRIVED WQKILMVRSL VVSPHEDMRT
 301 WLKYASLCGK SGRLALAHKT LVLLLGVDPS RQLDHPLPTV HPQVTYAYMK NMWKSARKID
 361 AFQHMQHFVQ TMQQQAQHAI ATEDQQHKQE LHKLMARCFL KLGEWQLNLQ GINESTIPKV
 421 LQYYSAATEH DRSWYKAWHA WAVMNFEAVL HYKHQNQARD EKKKLRHASG ANITNATTAA
 481 TTAATATTTA STEGSNSESE AESTENSPTP SPLQKKVTED LSKTLLMYTV PAVQGFFRSI
 541 SLSRGNNLQD TLRVLTLWFD YGHWPDVNEA LVEGVKAIQI DTWLQVIPQL IARIDTPRPL
 601 VGRLIHQLLT DIGRYHPQAL IYPLTVASKS TTTARHNAAN KILKNMCEHS NTLVQQAMMV
 661 SEELIRVAIL WHEMWHEGLE EASRLYFGER NVKGMFEVLE PLHAMMERGP QTLKETSFNQ
 721 AYGRDLMEAQ EWCRKYMKSG NVKDLTQAWD LYYHVFRRIS KQLPQLTSLE LQYVSPKLLM
 781 CRDLELAVPG TYDPNQPIIR IQSIAPSLQV ITSKQRPRKL TLMGSNGHEF VFLLKGHEDL
 841 RQDERVMQLF GLVNTLLAND PTSLRKNLSI QRYAVIPLST NSGLIGWVPH CDTLHALIRD
 901 YREKKKILLN IEHRIMLRMA PDYDHLTLMQ KVEVFEHAVN NTAGDDLAKL LWLKSPSSEV
 961 WFDRRTNYTR SLAVMSMVGY ILGLGDRHPS NLMLDRLSGK ILHIDFGDCF EVAMTREKFP
1021 EKIPFRLTRM LTNAMEVTGL DGNYRITCHT VMEVLREHKD SVMAVLEAFV YDPLLNWRLM
1081 DTNTKGNKRS RTRTDSYSAG QSVEILDGVE LGEPAHKKTG TTVPESIHSF IGDGLVKPEA
1141 LNKKAIQIIN RVRDKLTGRD FSHDDTLDVP TQVELLIKQA TSHENLCQCY IGWCPFW
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Recombinant mTOR nucleotide sequence:

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   1 atggactaca aagacgatga cgacaaggaa ttcatggaac acagtgacaa gggccccctg
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 121 tatgccaaag cactacacta caaagaactg gagttccaga aaggccccac ccctgccatt
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 241 ttagaatatg ccatgaaaca ctttggagag ctggagatcc aggctacctg gtatgagaaa
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 481 gcccggatgg ctgctgcagc tgcatggggt ttaggtcagt gggacagcat ggaagaatac
 541 acctgtatga tccctcggga cacccatgat ggggcatttt atagagctgt gctggcactg
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