

# Certificate of Analysis

**ACTR2, active**  
**(Recombinant enzyme expressed in Sf21 insect cells)**  
**Item # 16-009, 16-009-K, 16-009M**  
**Parent Lot # 227174**

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 GST-tagged, recombinant, human ACTR2 amino acids 162-end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione agarose. Purity 93% by SDS-PAGE and Coomassie blue staining. MW = 66 kDa.

**Specific Activity (Parent lot# 227174):** 73 U/mg, where one unit of ACTR2 activity is defined as 1 nmol phosphate incorporated into 2 mg/ml casein per minute at 30°C with a final ATP concentration of 100 µM.

**Formulation:** 1.16 mg/ml of enzyme in 50 mM Tris/HCl pH 7.5, 300 mM NaCl, 0.1 mM EGTA, 0.03% Brij-35, 270 mM sucrose, 1 mM benzamidine, 0.2 mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

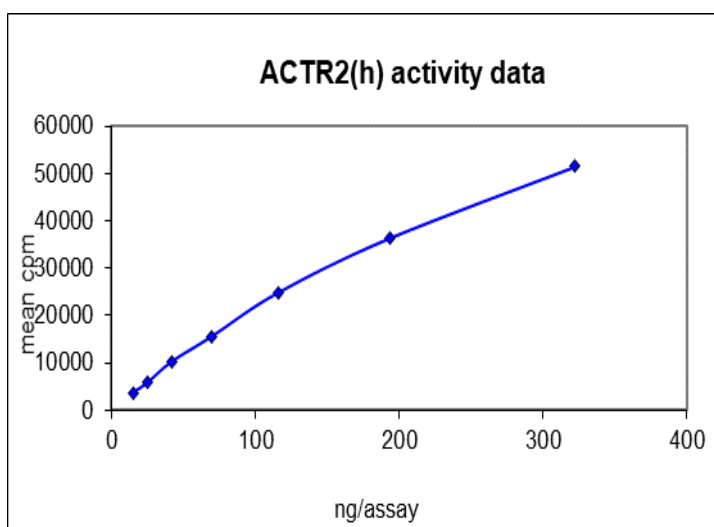
**Storage and Stability:** On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

**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.

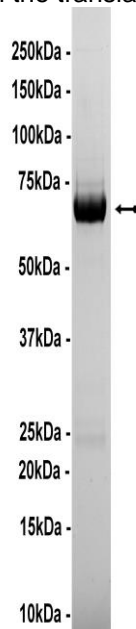
**FOR IN VITRO RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS OR ANIMALS**

## Quality Control Testing

**Kinase Assay:** 15–322 ng of this lot of enzyme phosphorylated 2 mg/ml casein 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 ACTR2 with the translated sequence listed on page three.



**SDS-PAGE and Coomassie Stain:** Purity was assessed by SDS-PAGE and Coomassie blue staining using 3 µg of ACTR2, active.

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### Kinase Assay Protocol

#### Stock Solutions:

- 5 x Reaction Buffer:** 100 mM MES pH 6.0, 1 mM EDTA,
- Casein:** Use at a final assay concentration of 2 mg/ml. Prepare a 20 mg/ml stock and add 2.5  $\mu$ l of stock per assay point.
- Manganese chloride:** Use at a final assay concentration of 2 mM. Prepare a 100 mM stock and add 0.5  $\mu$ l per assay point.
- Sodium chloride:** Use at a final assay concentration of 20 mM. Prepare a stock and add 0.17  $\mu$ l per assay point.
- ACTR2, active:** Dilute with 20 mM MES pH 6.0, 1 mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1 mg/ml BSA. Use 15–322 ng per assay point.
- $[\gamma\text{-}^{33}\text{P}]\text{ATP}$ :** 2.5 x MgAc/ $[\gamma\text{-}^{33}\text{P}]\text{ATP}$  cocktail: 25 mM MgAc and 0.25 mM ATP to which is added  $[\gamma\text{-}^{33}\text{P}]\text{ATP}$  (specific activity approximately 500 – 800 cpm/pmol as required).

#### Assay Procedure (96 well plate format):

- Add 5  $\mu$ l of 5 x reaction buffer per assay to wells.
- Add 4.33  $\mu$ l of dH<sub>2</sub>O
- Add 2.5  $\mu$ l of casein.
- Add 0.5  $\mu$ l of manganese chloride
- Add 0.17  $\mu$ l of sodium chloride
- Add **2.5  $\mu$ l (15–322 ng) ACTR2, active.**
- Add 10  $\mu$ l of diluted  $[\gamma\text{-}^{33}\text{P}]\text{ATP}$  mixture.
- Incubate for 10 minutes at 30°C.
- Stop the reaction by adding 5  $\mu$ l of 3% phosphoric acid.
- Transfer a 10  $\mu$ l aliquot onto the appropriate area of a **P30 Filtermat.**
- Wash the filtermat three times for 5 minutes with 75 mM phosphoric acid.
- Wash the filtermat once for 2 minutes with methanol.
- Transfer the filtermat to a sealable plastic bag and add 4 ml of scintillation cocktail.
- Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1  $\mu$ l of 30% phosphoric acid.

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## ACTR2, active Sequence Information

<b><u>Protein</u></b>	Human ACTR2
<b><u>Tags</u></b>	N-terminal GST
<b><u>Native sequence</u></b>	R230 of the recombinant protein is equivalent to R162 of human ACTR2
<b><u>Accession number</u></b>	GenBank NM_001616.2

### Recombinant ACTR2 amino acid sequence:

```

1  MSPILGYWKI  KGLVQPTRLL  LEYLEEKYEE  HLYERDEGDK  WRNKKFELGL  EFPNLPYYID
61  GDVKLTQSMA  IIRYIADKHN  MLGGCPKERA  EISMLEGAVL  DIRYGVSRIA  YSKDFETLKV
121  DFSLKLP EML  KMFEDRLCHK  TYLNGDHVTH  PDFMLYDALD  VVLYMDPMCL  DAFPKLVCFK
181  KRIEAI PQID  KYLKSSKYIA  WPLQGQWATF  GGGDHPPKSD  LVPRGSKEFR  HHKMAYPPVL
241  VPTQDPGPPP  PSPLLGLKPL  QLLEVKARGR  FGCVWKAQLL  NEYVAVKIFP  IQDKQSWQNE
301  YEVYSLPGMK  HENILQFIGA  EKRGTSDVD  LWLITAFHEK  GSLSDFLKAN  VVSWNELCHI
361  AETMARGLAY  LHEDI PGLKD  GHKPAISHRD  IKSKNVLLKN  NLTACIADFG  LALKFEAGKS
421  AGDTHGQVGT  RRYMAPEVLE  GAINFQRDAF  LRIDMYAMGL  VLWELASRCT  AADGPVDEYM
481  LPFEEEIGQH  PSLEDMQEVV  VHKKR PVL R  DYWQKHAGMA  MLCETIEECW  DHDAEARLSA
541  GCVGERITQM  QRLTNIITTE  DIVTVVTMVT  NVDFPPKESS  L

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### Recombinant ACTR2 nucleotide sequence:

```

1  atgtccccta  tactaggtta  ttggaaaatt  aagggccttg  tgcaaccac  tgcacttctt
61  ttggaatc  ttgaagaaa  atatgaagag  catttgtag  agcgcgatga  aggtgataaa
121  tggcgaaaca  aaaagtttga  attgggtttg  gagtttccca  atcttcctta  ttatattgat
181  ggtgatgta  aattaacaca  gtctatggcc  atcatacggt  atatagctga  caagcacaac
241  atgttgggtg  gttgtccaaa  agagcgtgca  gagatttcaa  tgcttgaagg  agcggttttg
301  gatattagat  acgggtgttc  gagaattgca  tatagtaaag  actttgaaac  tctcaaagtt
361  gattttctta  gcaagctacc  tgaaatgctg  aaaatggtcg  aagatcgttt  atgtcataaa
421  acatatttaa  atggtgatca  tgtaaccat  cctgacttca  tgttgatga  cgctcttgat
481  gttgttttat  acatggacc  aatgtgcctg  gatgcgttcc  caaaattagt  ttgttttaaa
541  aaacgtattg  aagctatccc  acaaattgat  aagtacttga  aatccagcaa  gtatatagca
601  tggcctttgc  agggctggca  agccacgttt  ggtggtggcg  accatcctcc  aaaatcggat
661  ctggttccgc  gtggatccaa  ggaattcagg  catcacaaga  tggcctacc  tctgtactt
721  gttccaactc  aagaccagg  accacccca  ctttctccat  tactaggttt  gaaaccactg
781  cagttattag  aagtgaaagc  aaggggaaga  tttggttggt  tctggaaagc  ccagttgctt
841  aacgaatag  tggctgtcaa  aatatttcca  atacaggaca  aacagtcag  gcaaaatgaa
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1201  aacctgacag  cttgcattgc  tgactttggg  ttggccttaa  aatttgaggc  tggcaagtct
1261  gcaggcgata  cccatggaca  ggttggtacc  cggagggtaca  tggctccaga  ggtattagag
1321  ggtgctataa  acttccaaag  ggatgcatt  ttgaggatag  atatgtatgc  catgggatta
1381  gtcctatggg  aactggcttc  tcgctgtact  gctgcagatg  gacctgtaga  tgaatacatg
1441  ttgccatttg  aggaggaat  tggccagcat  ccatctcttg  aagacatgca  ggaagttggt

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## Certificate of Analysis

```
1501 gtgcataaaa aaaagaggcc tgttttaaga gattattggc agaaacatgc tggaatggca
1561 atgctctgtg aaaccattga agaatgttgg gatcacgacg cagaagccag gttatcagct
1621 ggatgtgtag gtgaaagaat taccagatg cagagactaa caaatattat taccacagag
1681 gacattgtaa cagtggtcac aatggtgaca aatgttgact ttcctcccaa agaattctagt
1741 ctatga
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Reviewed and approved by site quality representative.

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