

Certificate of Analysis

MuSK, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-634, 14-634-K, 14-634M

Parent Lot # WAB0509

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 6His-tagged, recombinant, human MuSK, amino acids 530–end, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺-NTA agarose. Purity 82% by SDS-PAGE and Coomassie blue staining. MW = 43.1kDa.

Specific Activity (Parent lot# WAB0509): 78U/mg, where one unit of MuSK, active activity is defined as 1nmol phosphate incorporated into 0.33mg/ml myelin basic protein (MBP) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.09mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1 % 2-mercaptoethanol. Frozen solution.

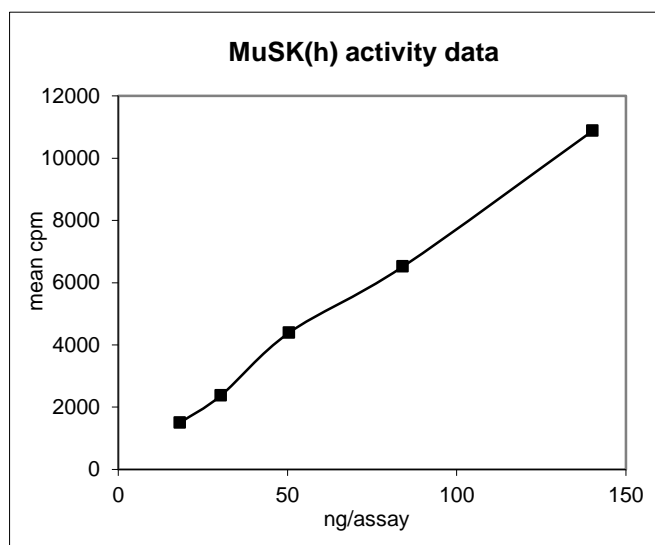
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

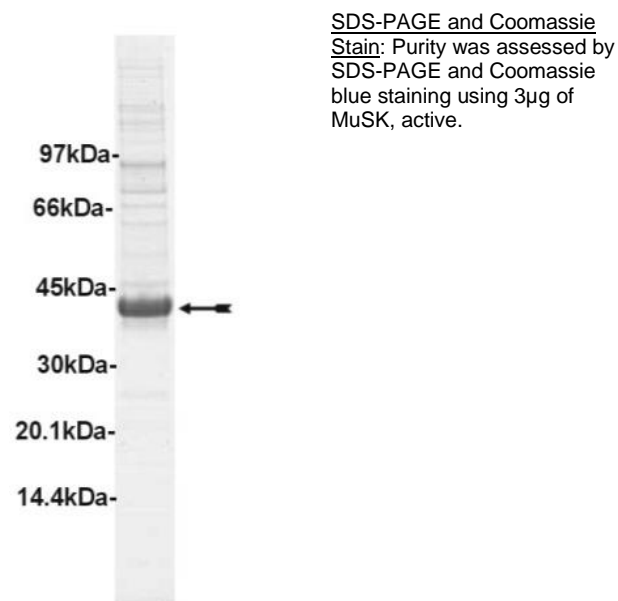
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 18–140ng of this lot of enzyme phosphorylated 0.33 mg/ml myelin basic protein (MBP) 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 MuSK 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. **Myelin Basic Protein (MBP):** Use at a final assay concentration of 0.33mg/ml. Make up a 3.3mg/ml stock. Use 2.5µl of stock per assay point.
3. **Manganese Chloride (MnCl₂):** Use at a final concentration of 5mM. Make up a 50mM stock. Use 2.5 µl of stock per assay point.
4. **MuSK, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 18–140ng per assay point.
5. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]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 **myelin basic protein (MBP)**.
3. Add **2.5µl (18–140ng) MuSK, active**.
4. Add 2.5µl of 50mM MnCl₂.
5. Add 2.5µl of dH₂O.
6. Add 10µl of diluted [γ-³³P]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 **P30 Filtermat**.
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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MuSK Sequence Information

Protein	MuSK
Tags	N-terminal 6His
Native sequence	E37 of the recombinant protein is equivalent to E530 of native human MuSK
Accession number	GenBank NM_005592

Recombinant MuSK amino acid sequence:

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1 MSYYHHHHHH DYDIPTTENL YFQGAMDPEF KGLRRLESAA VTLTTLPSSEL LLDRLHPNPM
61 YQRMPLLLNP KLLSLEYPRN NIEYVRDIGE GAFGRVFQAR APGLLPYEPF TMVAVKMLKE
121 EASADMQADF QREAALMAEF DNPNIKLLG VCAVGKPMCL LFEYMAVGDL NEFLRSMSPH
181 TVCSLSHSDL SMRAQVSSPG PPPLSCAEQL CIARQVAAGM AYLSEKRFVH RDLATRNCLV
241 GENMVKIAD FGLSRNIYSA DYYKANENDA IPIRWMPPEP IFYNRYTTES DVWAYGVVLW
301 EIFSYGLQPY YGMAHEEVIY YVRDGNILSC PENCPELYN LMRLCWSKLP ADRPSFTSIH
361 RILERMCERA EGTVS

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Recombinant MuSK nucleotide sequence:

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1 atgtcgtact accatcacca tcaccatcac gattacgata tcccaacgac cgaaaacctg
61 tattttcagg gcgcatgga tccggaattc aaaggcctac gtcgacttga atcagcagca
121 gtaaccctca ccacactgcc ttctgagctc ttactagata gacttcattc caaccccatg
181 taccagagga tgccgctcct tctgaacccc aaattgctca gcctggagta tccaaggaat
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