

Antibody Validation Report

aVR.MAP2K1.Q02750.AB_10981449.v3.1(December 16_2020)



A. Basic Target Information

Target Information

UniProt Accession Number: Q02750

Target Name: Dual specificity mitogen-activated protein kinase kinase 1 (MEK1)

Antibody Information

RRID: AB_10981449

Antibody Name: MEK1 Monoclonal Antibody (K.971.4)

Host Organism: Mouse

Clonality: Monoclonal

Vendor: Thermo

Catalog Number: MA5-15093

Lot Number: UL2904041A

Recombinant (Y/N): No

Organ/Tissue used for validation: A375

HuBMAP Platform Used: IP-MS

Protocols.io doi for Validation Protocol: 10.17504/protocols.io.bpmmmk46

ORCID ID of submitter: 0000-0002-5631-512X

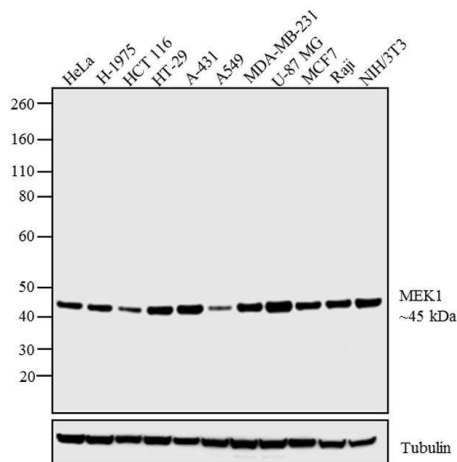
B. Validation Data

B.1. Vendor Validation: IP, WB

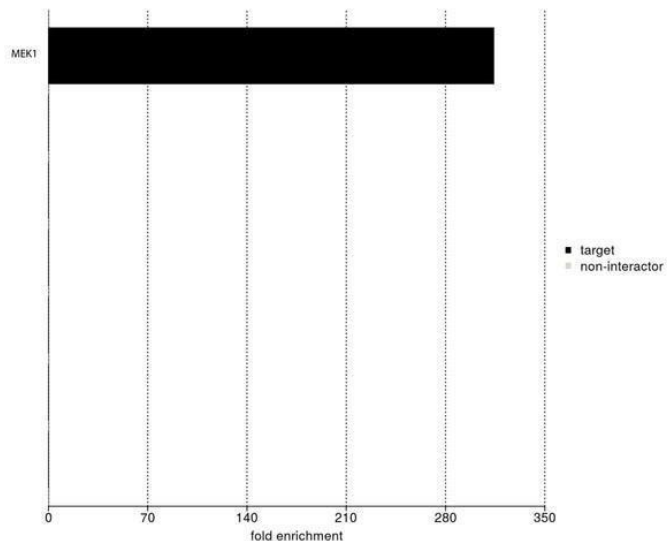
Date Accessed: 03/23/20

URL: <https://www.thermofisher.com/antibody/product/MEK1-Antibody-clone-K-971-4-Monoclonal/MA5-15093>

Western Blot

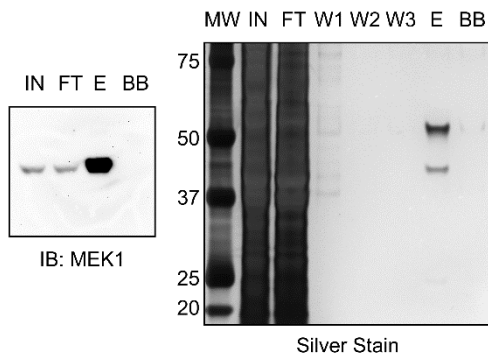


Immunoprecipitation and Mass Spectrometry

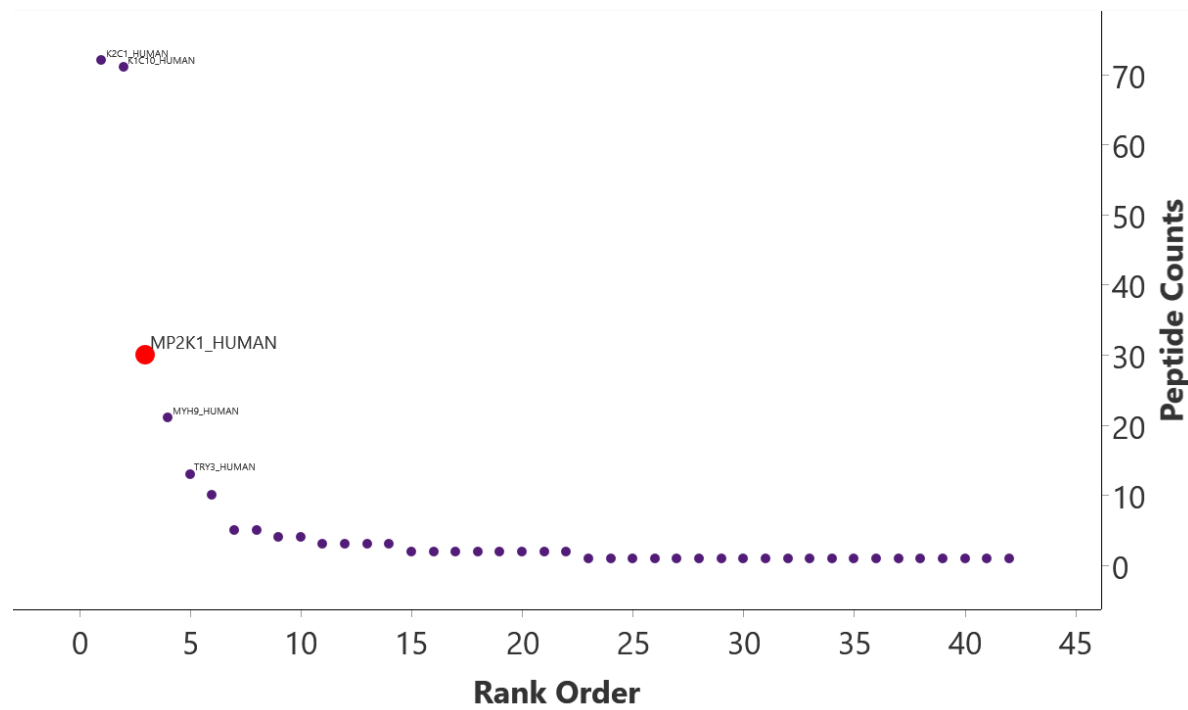


B.2. Laboratory Validation:

Immunoprecipitation/Western Blot



Immunoprecipitation/Bottom-Up MS



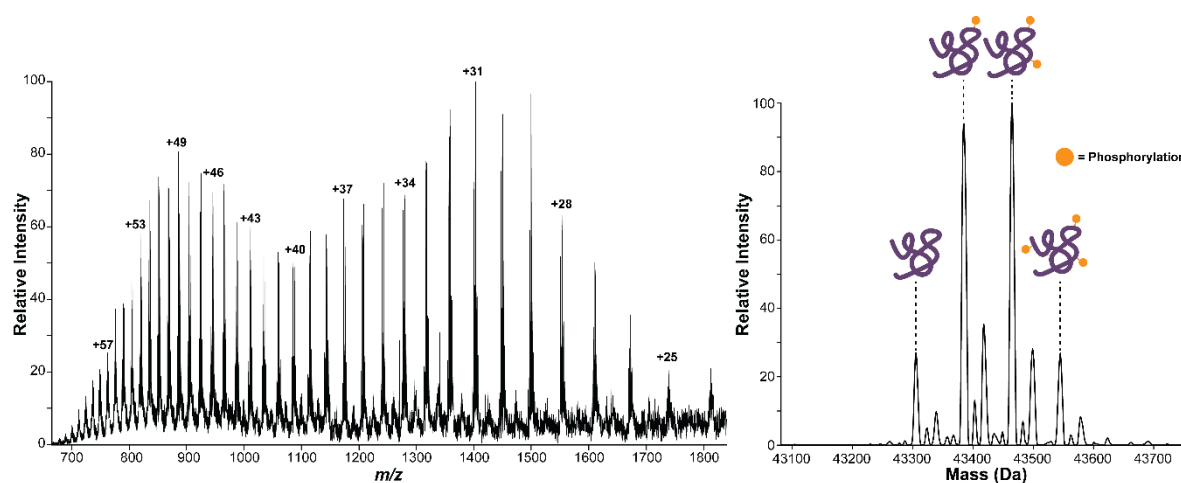
Immunoprecipitation/Top-Down MS

Proteoforms Identified:

1: PFR5430677, MEK1 Unmodified, 43,280.24 Da,
PKKKPTPIQLNPAPDGS AVNGTSSAETNLEALQKKLEELELDEQQRKRLEAFLTQKQKV
GELKDDDFEKISELGAGNGGVVFKVSHKPSGLVMARKLIHLEIKPAIRNQIIRELQVLHEC
NSPYIVGFYGA FYSDGEISICMEHMDGGSLDQVLKKAGRIPEQILGKVSI AVIKGLTYLRE
KHKIMHRDVKPSN ILVNSRGEIKLCDFGVSGQLIDSMANSFVGTRSYMSPERLQGTHYS
VQSDIWSMGLSLVEMAVGRYPIPPPPDAKELELMFGCQVEGDAAETPPRPRT PGRPLSS
YGMDSRPPMAIFELLDYIVNEPPPKLP SGVFSLEFQDFVNKCLIKNPAERADLKQLMVH
AFIKRSDAE EVDFA GWLCSTIGLNQPSTPTHAAGV

2: PFR5430678, MEK1 Phosphorylated, 43,360.21 Da,
 PKKKPTPIQLNPAPDGSVNGTSSAETNLEALQKKLEELDEQQQRKRLEAFLTQKQKV
 GELKDDDFEKISELGAGNGGVVFKVSHKPSGLVMARKLIHLEIKPAIRNQIIRELQVLHEC
 NSPYIVGFYGAIFYSDGEISICMEHMDGGSLDQVLKKAGRIPEQILGKVSIAVIKGLTYLRE
 KHKIMHRDVKPSNILVNSRGEIKLCDFGVSGQLIDSMANSFVGTRSYMSPERLQGTHYS
 VQSDIWSMGLSLVEMAVGRYPPIPPDAKELELMFGCQVEGDAAETPPRPRTPGRPLSS
 YGMDSRPPMAIFELLDYIVNEPPPKLP SGVFSLEFQDFVNKCLIKNPAERADLKQLMVH
 AFIKRSDAEEVDFAGWLCSTIGLNQPSTPTHAAGV

Intact Mass Profile



IP-TDMS Graphical Fragment Map of Unmodified MEK1

N P K K K P T P I Q L N P A P D G S A V N G T S S A 25
 26 E T N L E A L Q K K L E E L D E Q Q R K R L E 50
 51 A F L T Q K Q K V G E L K D D D F E K I S E L G A 75
 76 G N G G V V F K V S H K P S G L V M A R K L I H L 100
 101 E I K P A I R N Q I I R E L Q V L H E C N S P Y I 125
 126 V G F Y G A F Y S D G E I S I C M E H M D G G S L 150
 151 D Q V L K K A G R I P E Q I L G K V S I A V I K G 175
 176 L T Y L R E K H K I M H R D V K P S N I L V N S R 200
 201 G E I K L C D F G V S G Q L I D S M A N S F V G T 225
 226 R S Y M S P E R L Q G T H Y S V Q S D I W S M G L 250
 251 S L V E M A V G R Y P I P P P D A K E L E L M F G 275
 276 C Q V E G D A A E T P P R P R T P G R P L S S Y G 300
 301 M D S R P P M A I F E L L D Y I V N E P P P K L P 325
 326 S G V F S L E F Q D F V N K C L I K N P A E R A D 350
 351 L K Q L M V H A F I K R S D A E E V D F A G W L C 375
 376 S T I G L N Q L P S T P T H A A G V C

- Proteoform Characterization Score (PCS): 338
- P-score: 1.5×10^{-33}
- Fragment ions explained: 21%
- Backbone cleavages: 7%
- Proteoform Record Number (PFR): **5430677**

IP Top-down Mass Spectrometry Fragment Map Monophosphorylated MEK1

N P K K K P T P I Q L N P A P D G S A V N G T S S A 25
26 E T N L E A L Q K K L E E L E L D E Q Q R K R L E 50
51 A F L T Q K Q K V G E L K D D D F E K I S E L G A 75
76 G N G G V V F K V S H K P S G L V M A R K L I H L 100
101 E I K P A I R N Q I I R E L Q V L H E N S P Y I 125
126 V G F Y G A F Y S D G E I S I C M E H M D G G S L 150
151 D Q V L K K A G R I P E Q I L G K V S I A V I K G 175
176 L T Y L R E K H K I M H R D V K P S N I L V N S R 200
201 G E I K L C D F G V S G Q L I D S M A N S F V G T 225
226 R S Y M S P E R L Q G T H Y S V Q S D I W S M G L 250
251 S L V E M A V G R Y P I P P P D A K E L E L M F G 275
276 C Q V E G D A A E T P P R P R T P G R P L S S Y G 300
301 M D S R P P M A I F E L L D Y I V N E P P P K L P 325
326 S G V F S L E F Q D F V N K C L I K N P A E R A D 350
351 L K Q L M V H A F I K R S D A E E V D F A G W L C 375
376 S T I G L N Q L P S T P T H A A G V C

- P-score: 1.5×10^{-33}
- Proteoform Characterization Score (PCS): 337
- Fragment ions explained: 21%
- Backbone cleavages: 7%
- Proteoform Record Number (PFR): **5430678**