Antibody Validation Report

aVR.PDZK1IP1.Q13113.AB_2877669.v1.0 (December 22_2020)

A. Basic Target Information

Target Information

UniProt Accession Number: Q13113
Target Name: PDZK1-interacting protein 1

Antibody Information

RRID: AB_2877669
Antibody Name: Recombinant Anti-Map17 antibody [EPR16827]
Host Organism: Rabbit
Clonality: Monoclonal
Vendor: AbCam
Catalog Number: ab199540
Lot Number: GR208212-2
Recombinant (Y/N): Yes

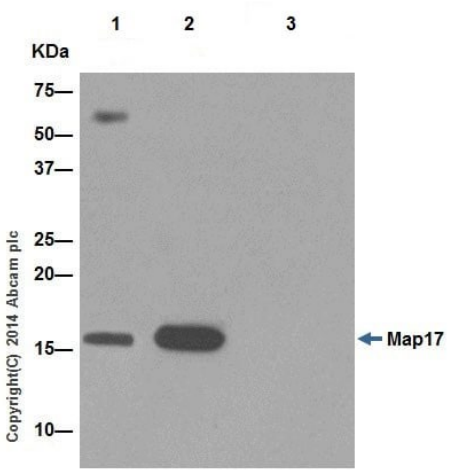
Organ/Tissue used for validation: Human Kidney
HuBMAP Platform Used: IP-MS
Protocols.io doi for Validation Protocol: 10.17504/protocols.io.bradm2a6
ORCID ID of submitter: 0000-0002-4549-2585

B. Validation Data

B.1. Vendor Validation: IP, WB

Date Accessed: 11/13/20

URL: https://www.abcam.com/map17-antibody-epr16827-ab199540.html



Map17 was immunoprecipitated from 1mg of Human fetal kidney whole cell lysate with ab199540 at 1/120 dilution. Western blot was performed from the immunoprecipitate using ab199540 at 1/1000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: Human fetal kidney whole cell lysate 10ug (Input). Lane 2: ab199540 IP in Human fetal kidney whole cell lysate. Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab199540 in Human fetal kidney whole cell lysate.

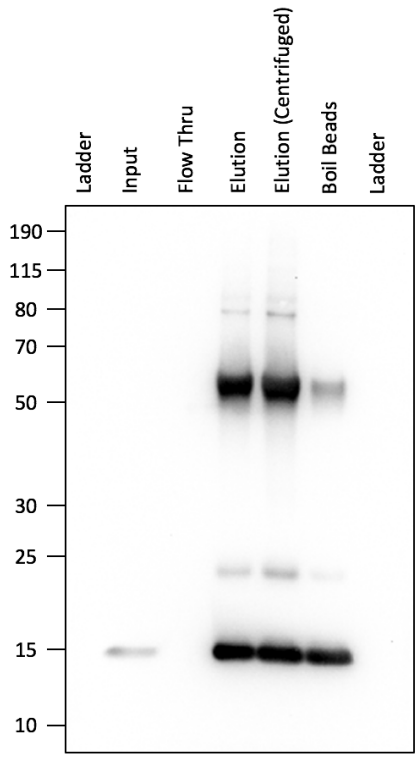
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.

B.2. Laboratory Validation:

Immunoprecipitation/Western Blot

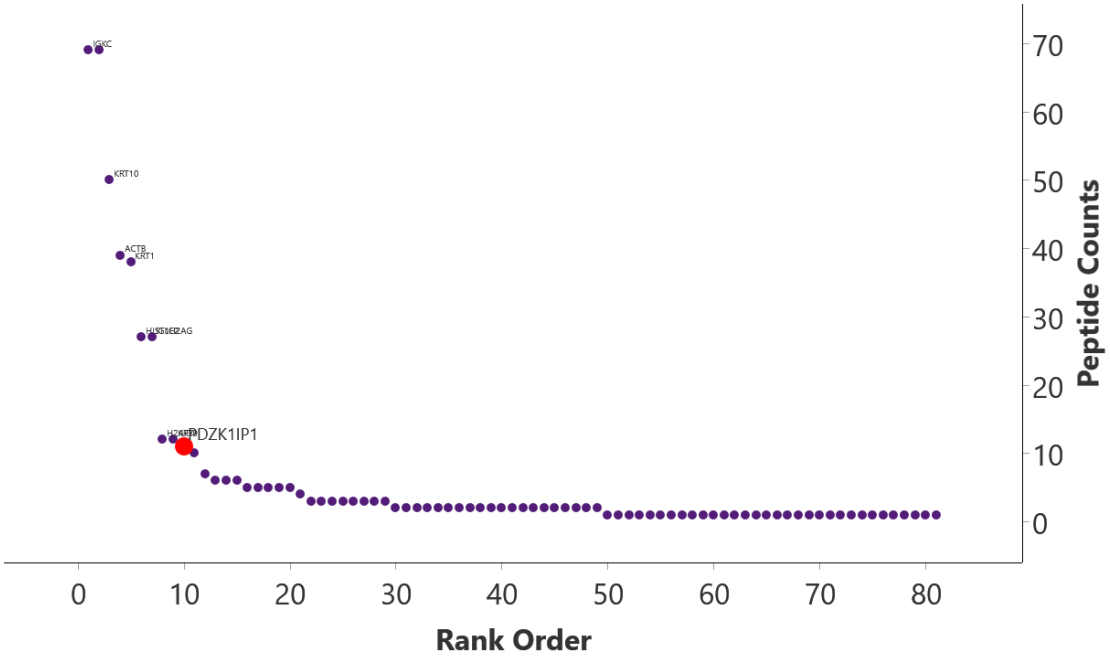
Map17 Immunoprecipitation for Top-Down Mass Spectrometry (abcam ab199540)



Primary: Rb mAB to Map17 (abcam ab199540) (1:10000)

Secondary: Goat anti-Rabbit (1:5000)

Immunoprecipitation/Bottom-Up MS

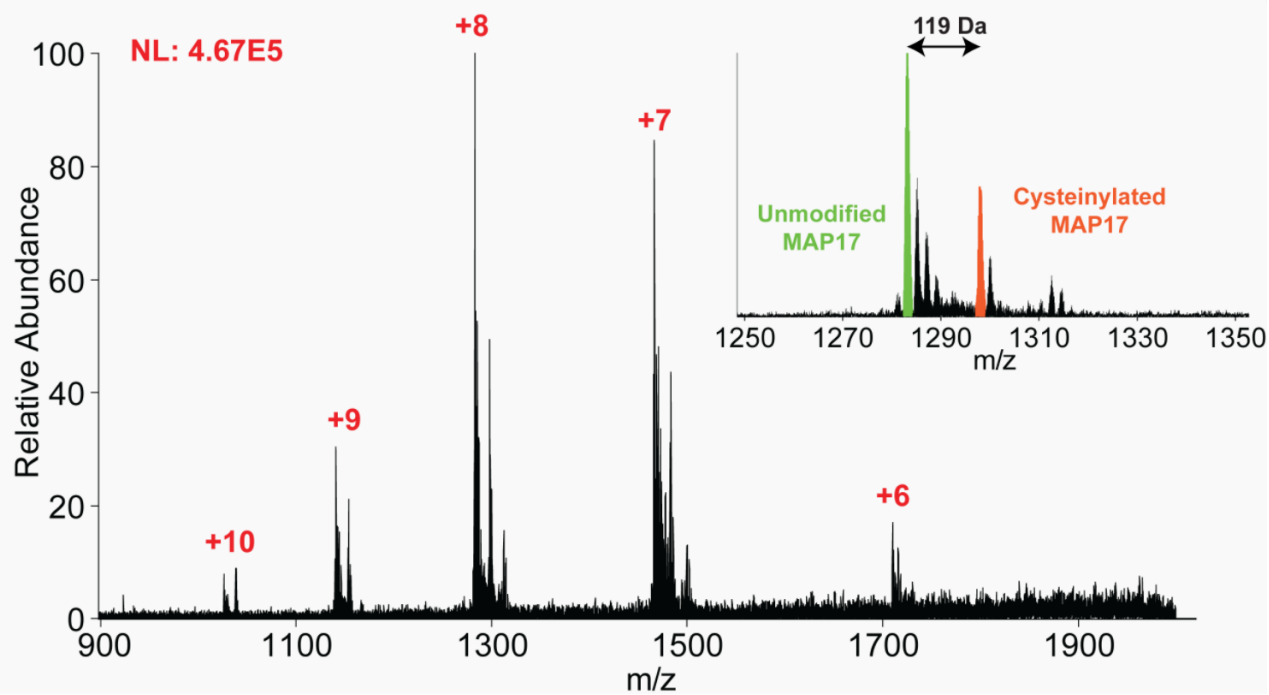


Immunoprecipitation/Top-Down MS

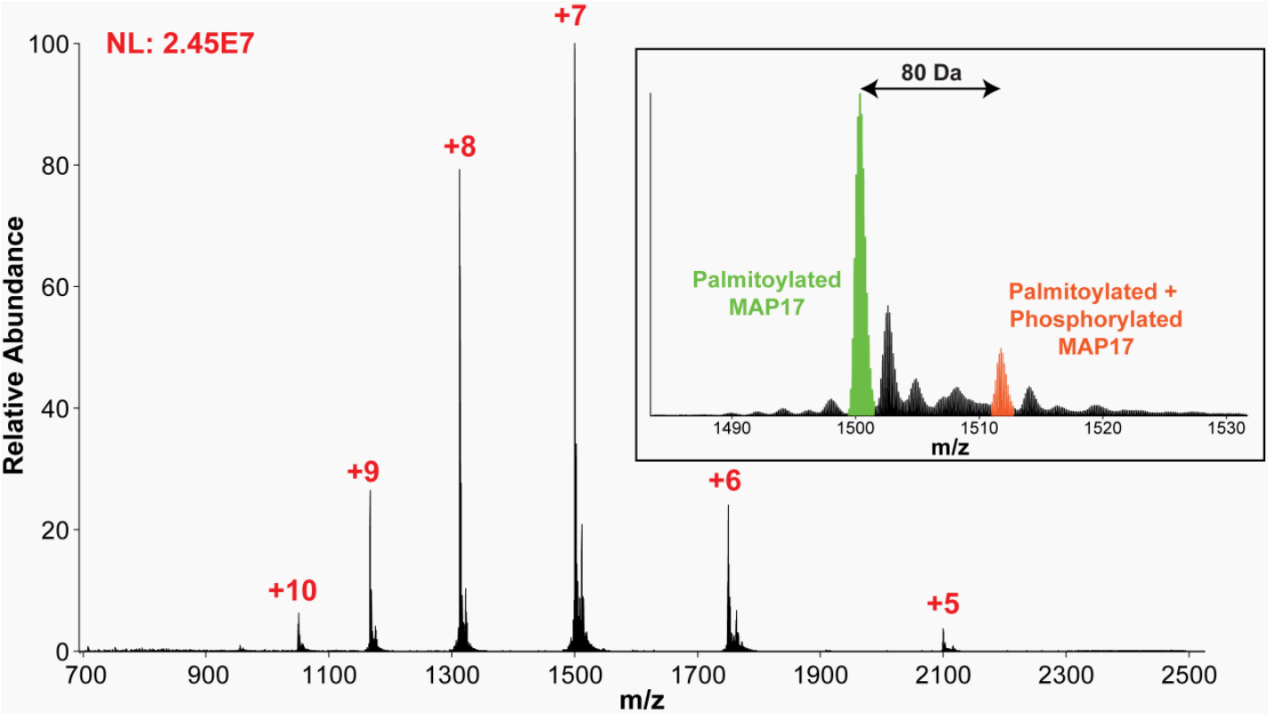
Proteoforms Identified:

1. PFR00005647342, PDZK1IP1-NTerm PyroQ, 10251.00 Da, [MOD:00040]-QQGLGNLQPWMQGLIAVAVFLVLVAIAFAVNHFWCQEEPEPAHMILTVGNKADGVLVGTDGRYSSMAASFRSSEHENAYENVPEEEGKVRSTPM
2. PFR00005647343, PDZK1IP1-NTerm PyroQ-Palmitoyl, 10489.22 Da, [MOD:00040]-QQGLGNLQPWMQGLIAVAVFLVLVAIAFAVNHFWC[MOD:00115]QEEPEPAHMILTVGNKADGVLVGTDGRYSSMAASFRSSEHENAYENVPEEEGKVRSTPM
3. PFR00005647344, PDZK1IP1-NTerm PyroQ-Palmitoyl-Phospho, 10569.19 Da, [MOD:00040]-QQGLGNLQPWMQGLIAVAVFLVLVAIAFAVNHFWC[MOD:00115]QEEPEPAHMILTVGNKADGVLVGTDGRYS[MOD:00046]SMAASFRSSEHENAYENVPEEEGKVRSTPM
4. PFR00005647345, PDZK1IP1-NTerm PyroQ-Cysteinyl, 10370.00 Da, [MOD:00040]-QQGLGNLQPWMQGLIAVAVFLVLVAIAFAVNHFWC[MOD:00765]QEEPEPAHMILTVGNKADGVLVGTDGRYSSMAASFRSSEHENAYENVPEEEGKVRSTPM

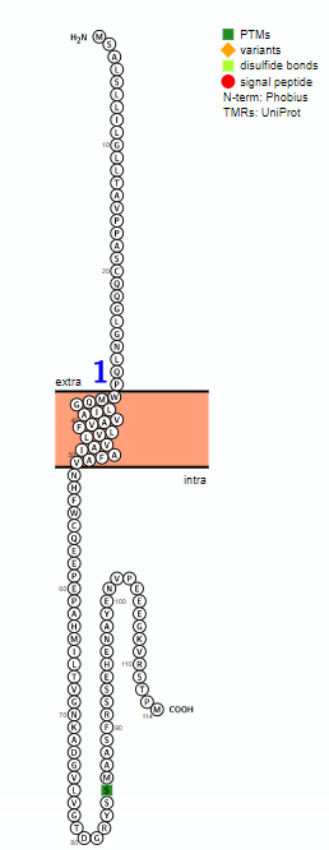
Intact Mass Profile
Proteoforms 1 and 4:



Proteoforms 2 and 3:

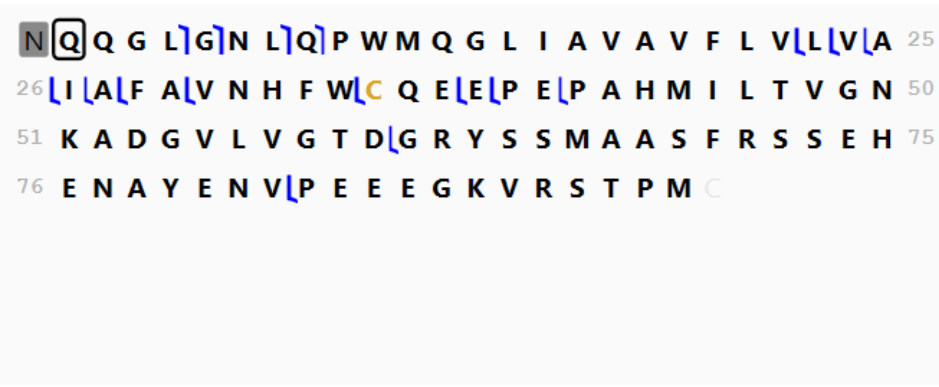


Protter (Omasits et al., Bioinformatics. 2013 Nov 21)



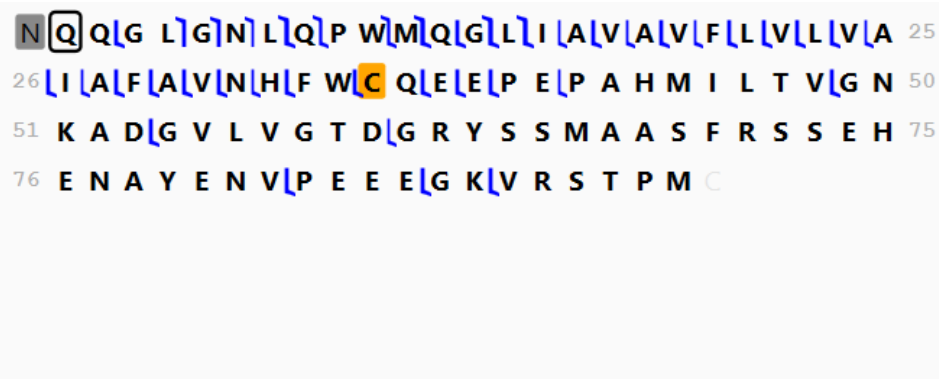
Supporting Information

Proteoform 1:



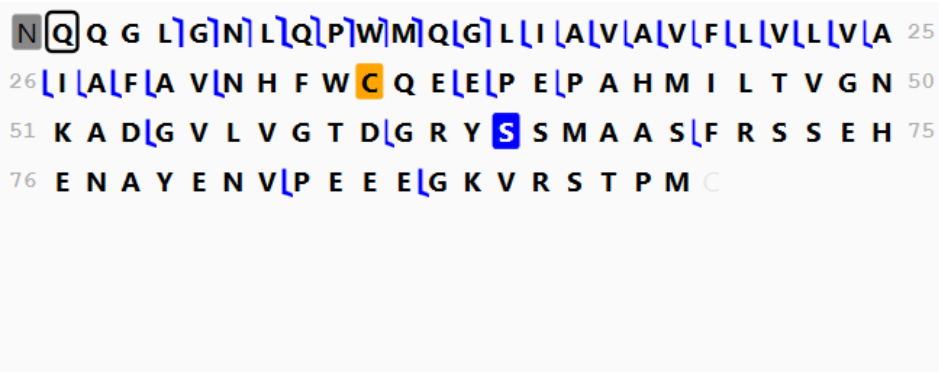
Precursor Mass	
Type:	Monoisotopic
Observed:	10,251.07
Theoretical:	10,251.00
Mass Diff. (Da):	0.071
Mass Diff. (ppm):	6.93
Scores	
PCS:	299.46
P-Score:	1.6e-30
% Fragments Expl...	40%
% Residue Cleava...	18%

Proteoform 2:



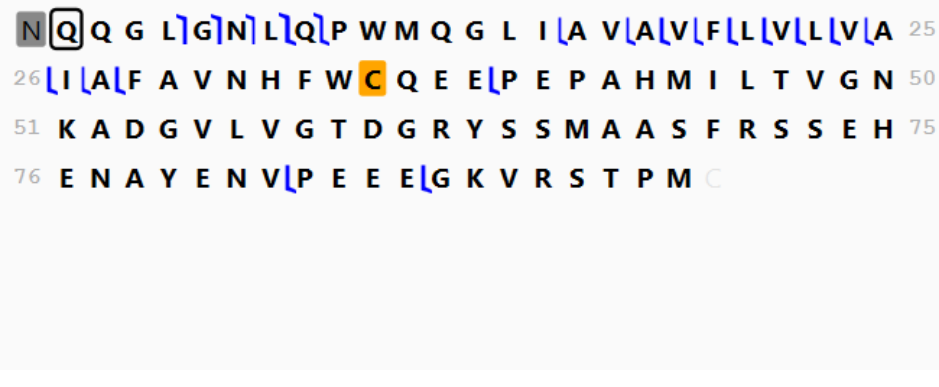
Precursor Mass	
Type:	Monoisotopic
Observed:	10,489.33
Theoretical:	10,489.22
Mass Diff. (Da):	0.103
Mass Diff. (ppm):	9.85
Scores	
PCS:	716.76
P-Score:	3.4e-63
% Fragments Expl...	35%
% Residue Cleava...	43%

Proteoform 3:



Precursor Mass	
Type:	Monoisotopic
Observed:	10,569.18
Theoretical:	10,569.19
Mass Diff. (Da):	-0.007
Mass Diff. (ppm):	-0.63
Scores	
PCS:	455.56
P-Score:	6.8e-43
% Fragments Expl...	23%
% Residue Cleava...	37%

Proteoform 4:



Precursor Mass	
Type:	Monoisotopic
Observed:	10,370.06
Theoretical:	10,370.00
Mass Diff. (Da):	0.057
Mass Diff. (ppm):	5.52
Scores	
PCS:	397.98
P-Score:	2.3e-38
% Fragments Expl...	43%
% Residue Cleava...	22%