

Antibody Validation Report

aVR.AQP1.P29972.AB_626694.v1.0 (November 2_2020)



A. Basic Target Information

Target Information

UniProt Accession Number: P29972

Target Name: Aquaporin-1

Antibody Information

RRID: AB_626694

Antibody Name: AQP1 (B-11) antibody

Host Organism: Mouse

Clonality: Monoclonal

Vendor: Santa Cruz

Catalog Number: sc-25287

Lot Number: B2820

Recombinant (Y/N): No

Organ/Tissue used for validation: Human Kidney

HuBMAP Platform Used: IP-MS

Protocols.io doi for Validation Protocol: 10.17504/protocols.io.bpfdmji6

ORCID ID of submitter: 0000-0002-8815-3372

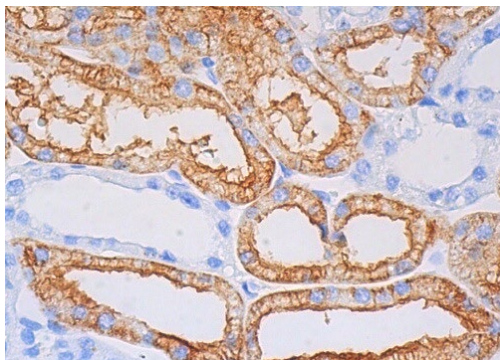
B. Validation Data

B.1. Vendor Validation: IP, WB, IF, IHC(P)

Date Accessed: 11/02/20

URL: <https://www.scbt.com/p/aqp1-antibody-b-11>

Immunohistochemistry (Formalin-fixed paraffin-embedded sections)



B.2. Laboratory Validation:

Immunoprecipitation/Western Blot

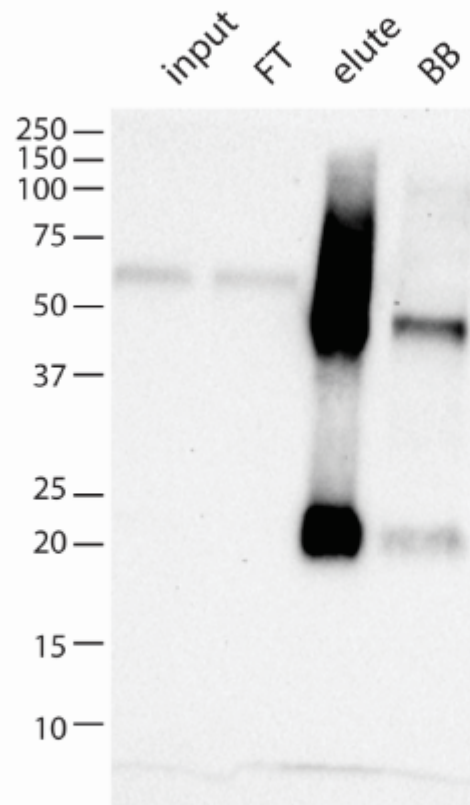
10/22/2020_Western blot: AQP1 IP_Kidney

4-20% Protean gel

AQP1 monoclonal Ab (sc-25287) 1:500 on 4C

Secondary anti-mouse IgG HRP (sc-2005) 1/5000 1 h RT

Amersham ECL WB detection reagents-RPN2209



IP conditions (manual):

20ug Ab sc-25287 (100ul) +120ul Protein A/G

1mg Kidney extract- Triton (28.8mg tissue)

3 washes in Triton Buffer

5 washes with TBS

1 Wash with H₂O

Elution 100ul 0.2%FA (20'RT and 10' at 37C)

Expected MW:

isoform 1: 28,526

isoform 2:19,956

isoform 3: 23,425

isoform 4:16,677

glycosylated: 35-45 kDa

Immunoprecipitation/Bottom-Up MS



- Note that there are very few tryptic peptides for AQP1

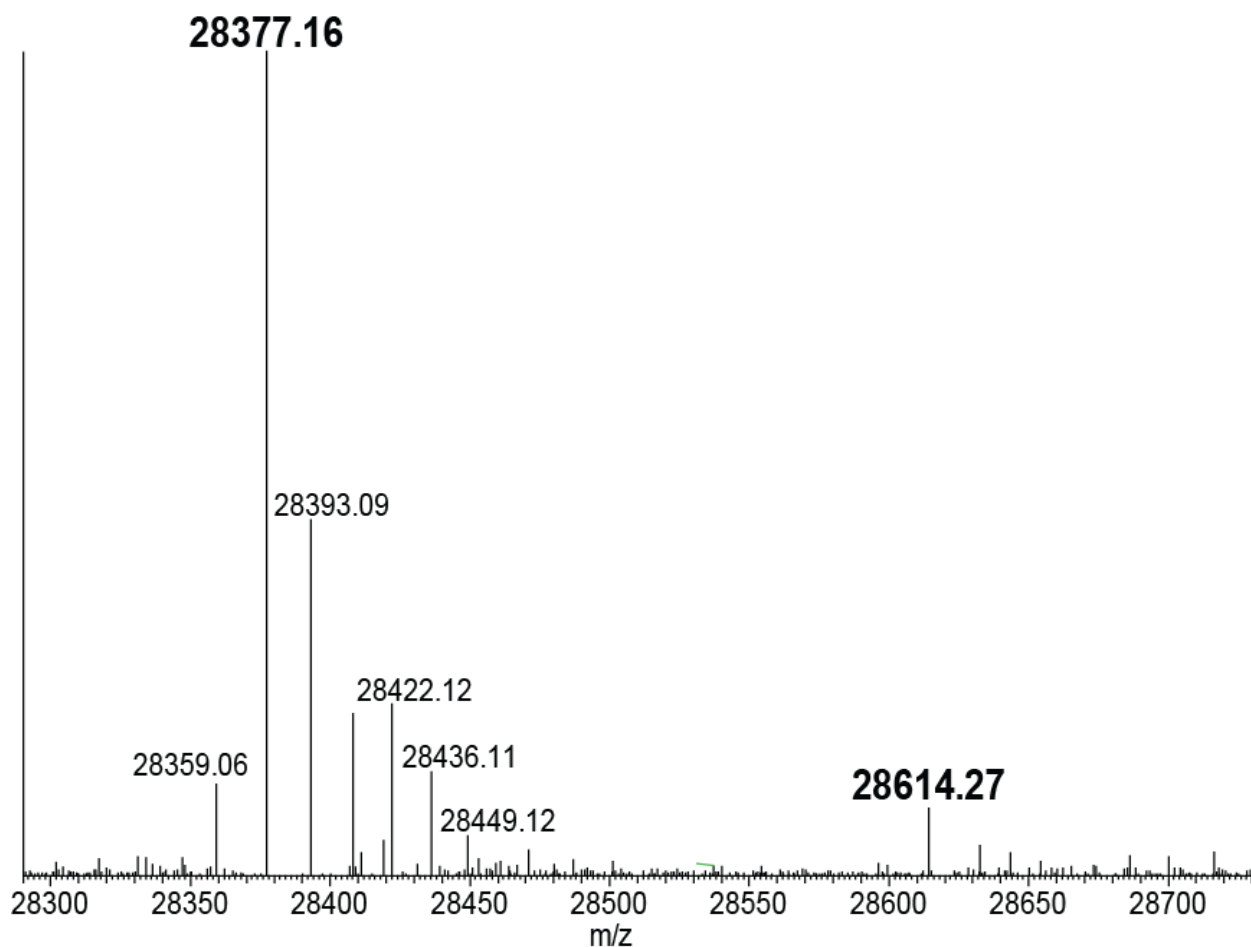
Immunoprecipitation/Top-Down MS

Proteoforms Identified:

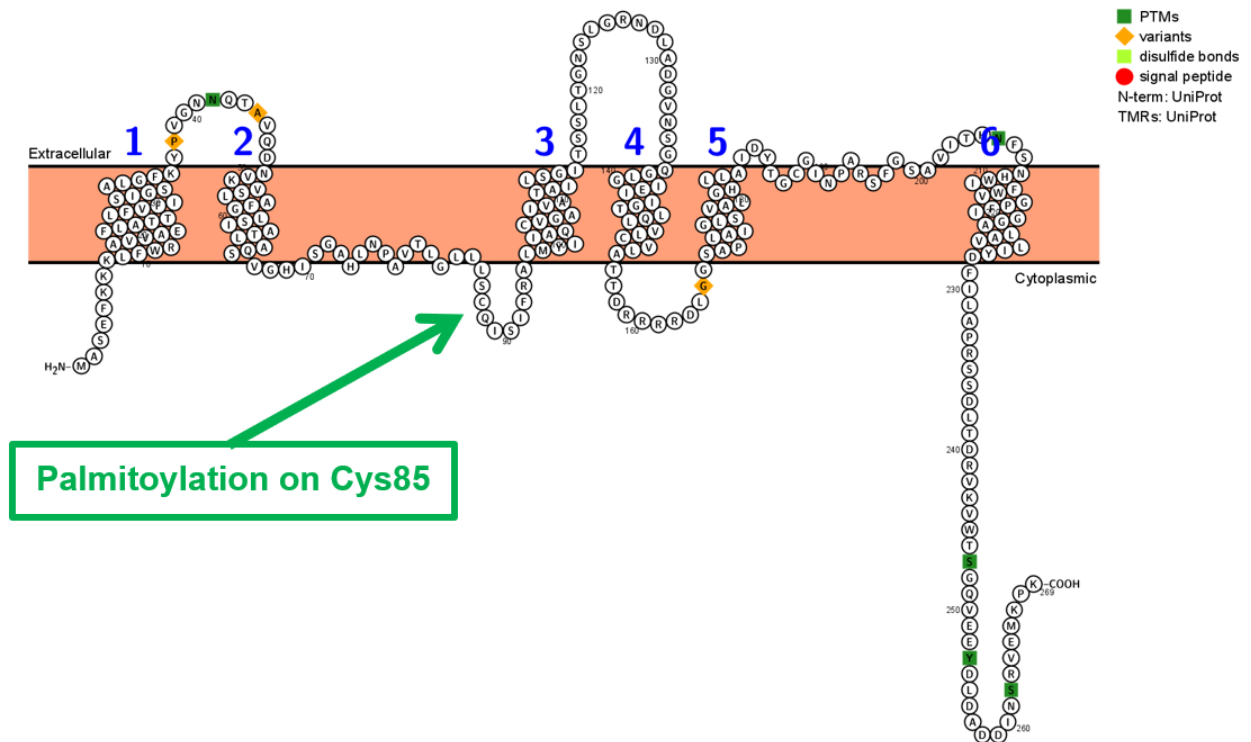
1: PFR5430674, Aquaporin 1, 28,377.16 Da,
ASEFKKKLFWRAVVAEFLATTLFVFISIGSALGFKYPVGNNQTAVQDNVKVSLAFGLSIAT
LAQSVGHISGAHLNPAVTLGLLLSCQISIFRALMYIIAQCVGAIVATAILSGITSSLTGNSLG
RNDLADGVNSGQGLGIEIIGTLQLVLCVLATTDRRRRDLGGSAPLAIGLSVALGHLLAIDY
TGCGINPARSFGSAVITHNFSNHWIFWVGPFIGGALAVLIYDFILAPRSSDLTDRVKVWTS
GQVEEYDL DADDINSRVEMKPK

2: PFR5430676, Palmitoylated Aquaporin 1, 28,615.38 Da,
ASEFKKKLFWRAVVAEFLATTLFVFISIGSALGFKYPVGNNQTAVQDNVKVSLAFGLSIAT
LAQSVGHISGAHLNPAVTLGLLLSC[MOD:00115]QISIFRALMYIIAQCVGAIVATAILSGIT
SSLTGNSLGRNDLADGVNSGQGLGIEIIGTLQLVLCVLATTDRRRRDLGGSAPLAIGLSV
ALGHLLAIDYTGCGINPARSFGSAVITHNFSNHWIFWVGPFIGGALAVLIYDFILAPRSSDL
TDRVKVWTSGQVEEYDL DADDINSRVEMKPK

Intact Mass Profile

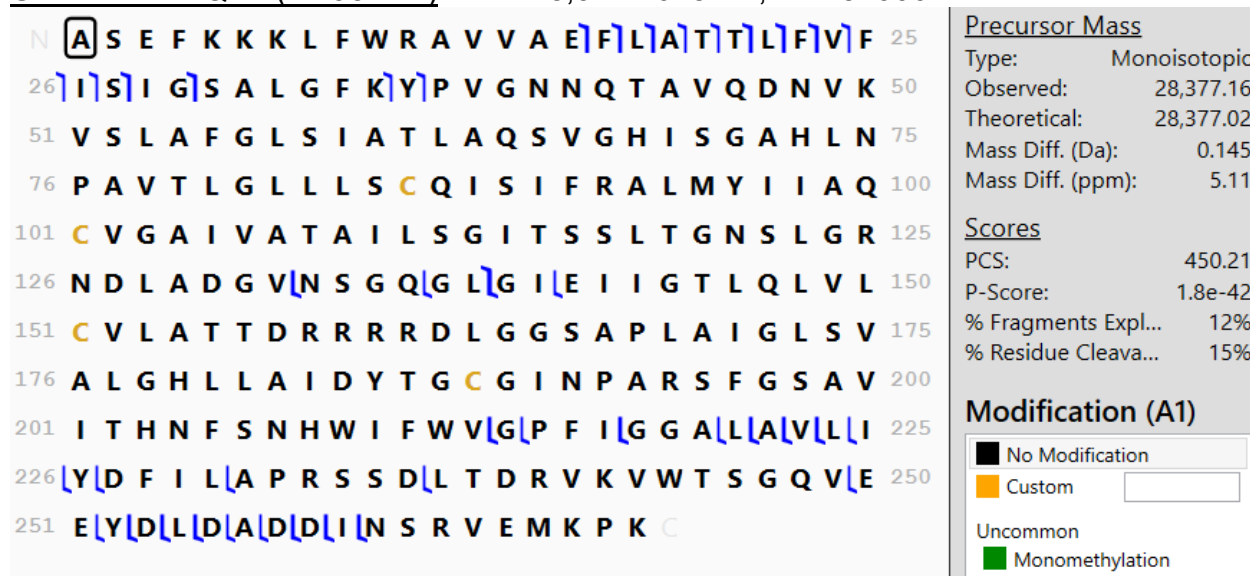


Protter (Omasits et al., Bioinformatics. 2013 Nov 21)

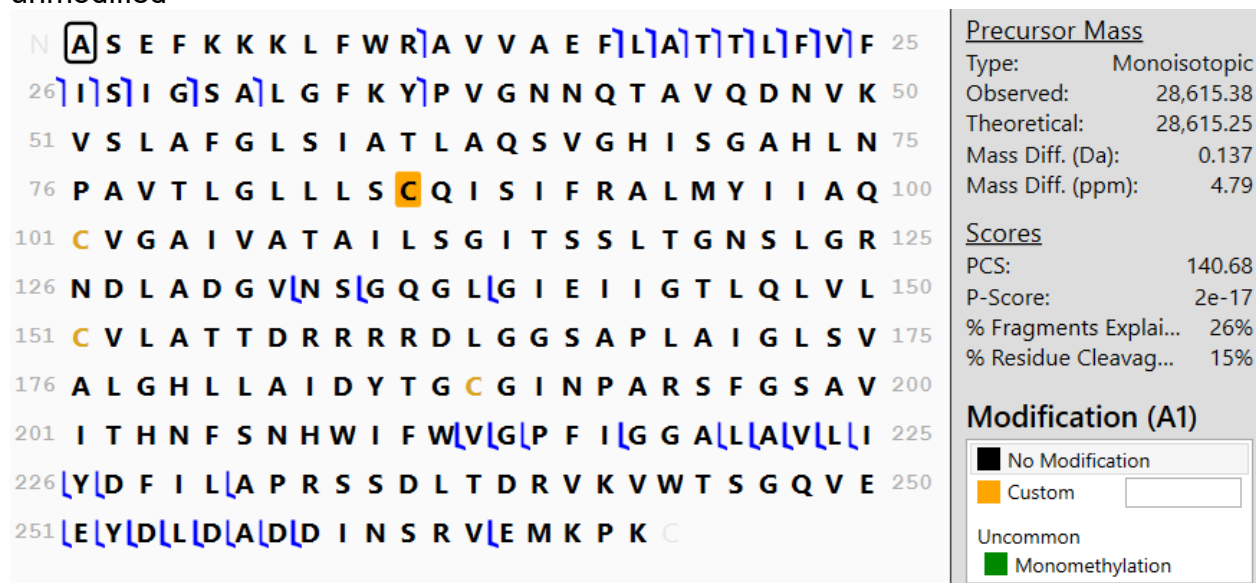


Backup Top Down Documentation

Unmodified AQP1 (17.98 min): MW: 28,377.1618 Da, PFR5430674

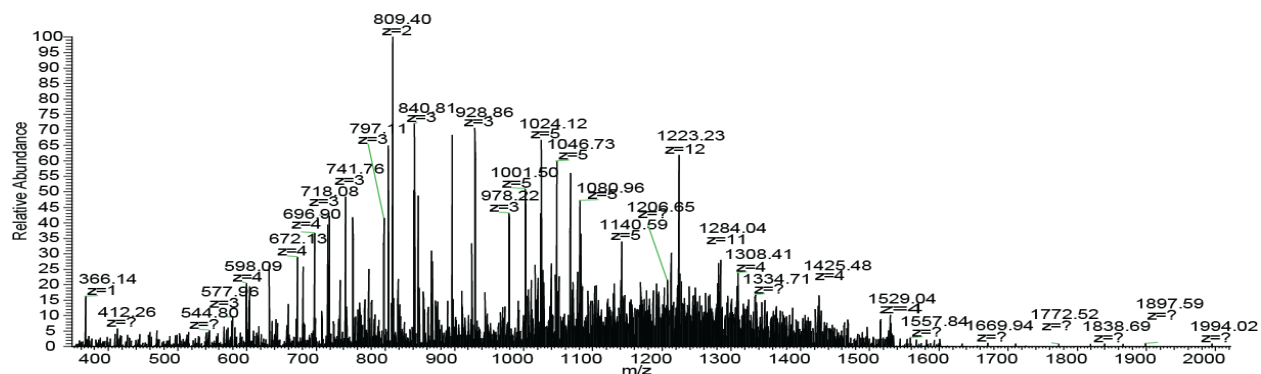
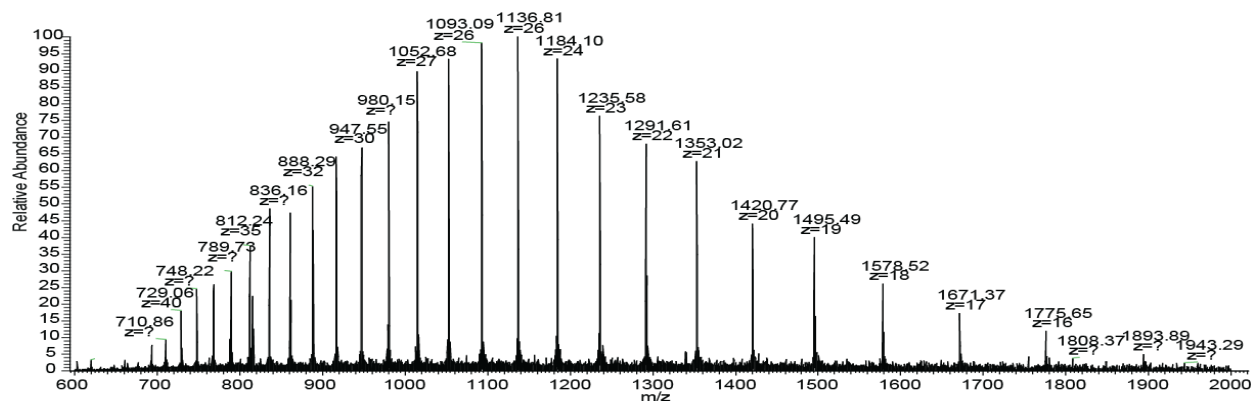


Palmitoylated AQP1 (21.31 min): MW: 28,615.3835 Da, PFR5430676, ~10% of unmodified



Palmitoylation tentatively localized to C86, 'medium confidence' per GPS-PALM.

Unmodified AQP1



Palmitoylated AQP1

