

## **Antibody Validation Report**

aVR.CDKN1A.P38936.AB\_10984461.v2.1 (November 3\_2020)



### **A. Basic Target Information**

#### **Target Information**

**UniProt Accession Number:** P38936

**Target Name:** Cyclin-dependent kinase inhibitor 1 (p21)

#### **Antibody Information**

**RRID:** AB\_10984461

**Antibody Name:** p21 Antibody (R.229.6)

**Host Organism:** Rabbit

**Clonality:** Monoclonal

**Vendor:** Thermo

**Catalog Number:** MA5-14949

**Lot Number:** VJ3094242

**Recombinant (Y/N):** No

**Organ/Tissue used for validation:** MCF7 Cell Line

**HuBMAP Platform Used:** IP-MS

**Protocols.io doi for Validation Protocol:** 10.17504/protocols.io.bq9pmz5n

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

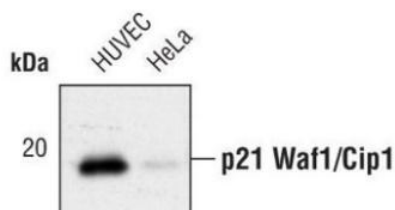
### **B. Validation Data**

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

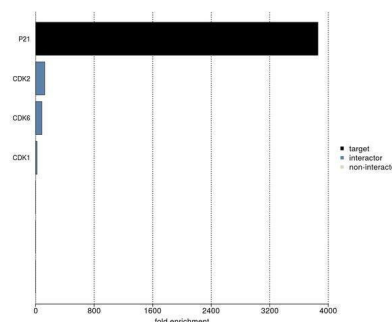
Date Accessed: 03/26/20

URL: <https://www.thermofisher.com/antibody/product/p21-Antibody-clone-R-229-6-Monoclonal/MA5-14949>

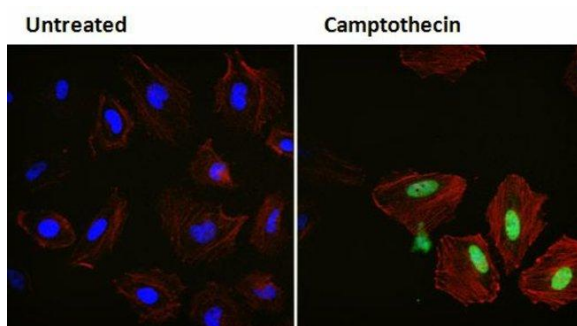
*Western Blot*



*Immunoprecipitation and Mass Spectrometry*



### Immunofluorescence

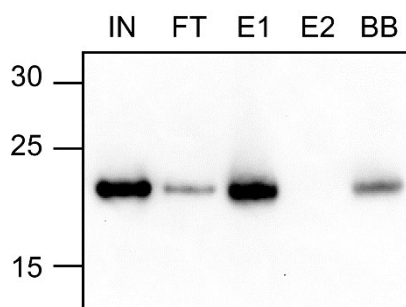


### IHC



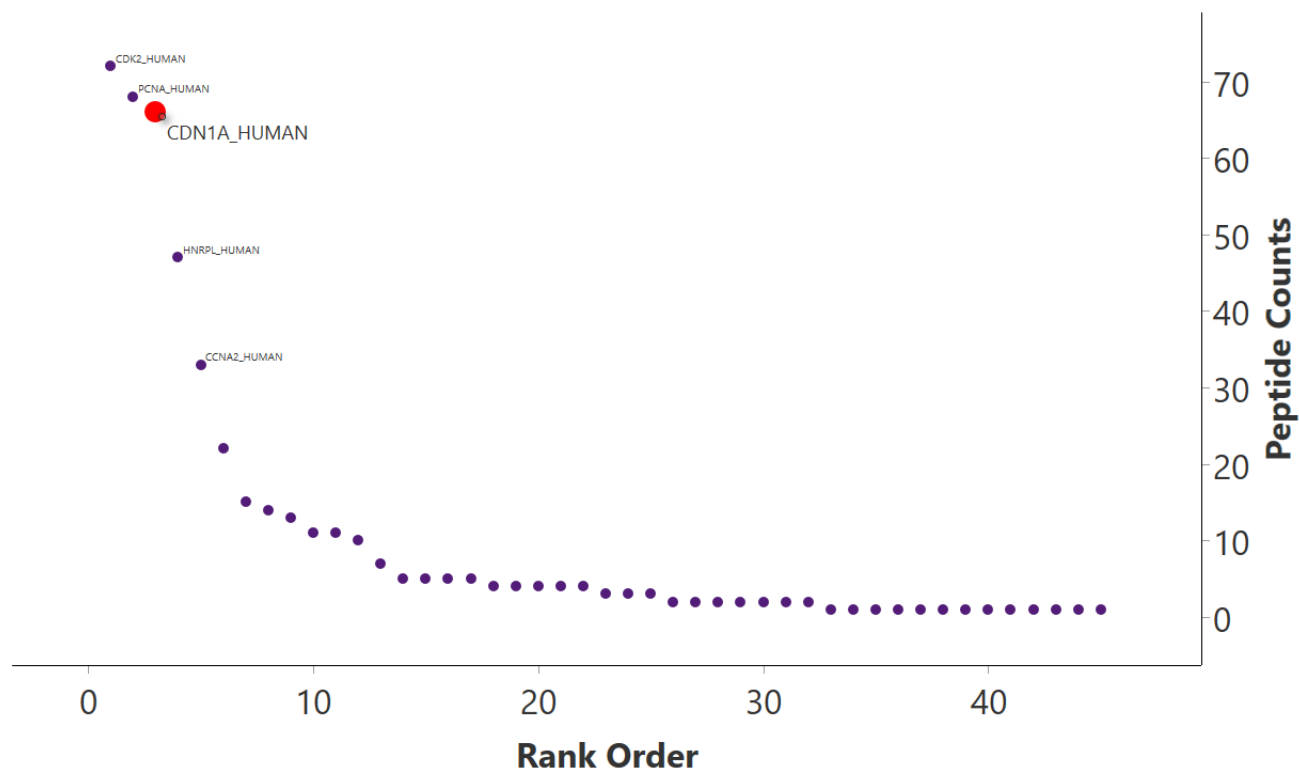
## B.2. Laboratory Validation:

### Immunoprecipitation/Western Blot



IP from MCF7 cells treated with 5  $\mu$ M nutlin-3a

### Immunoprecipitation/Bottom-Up MS



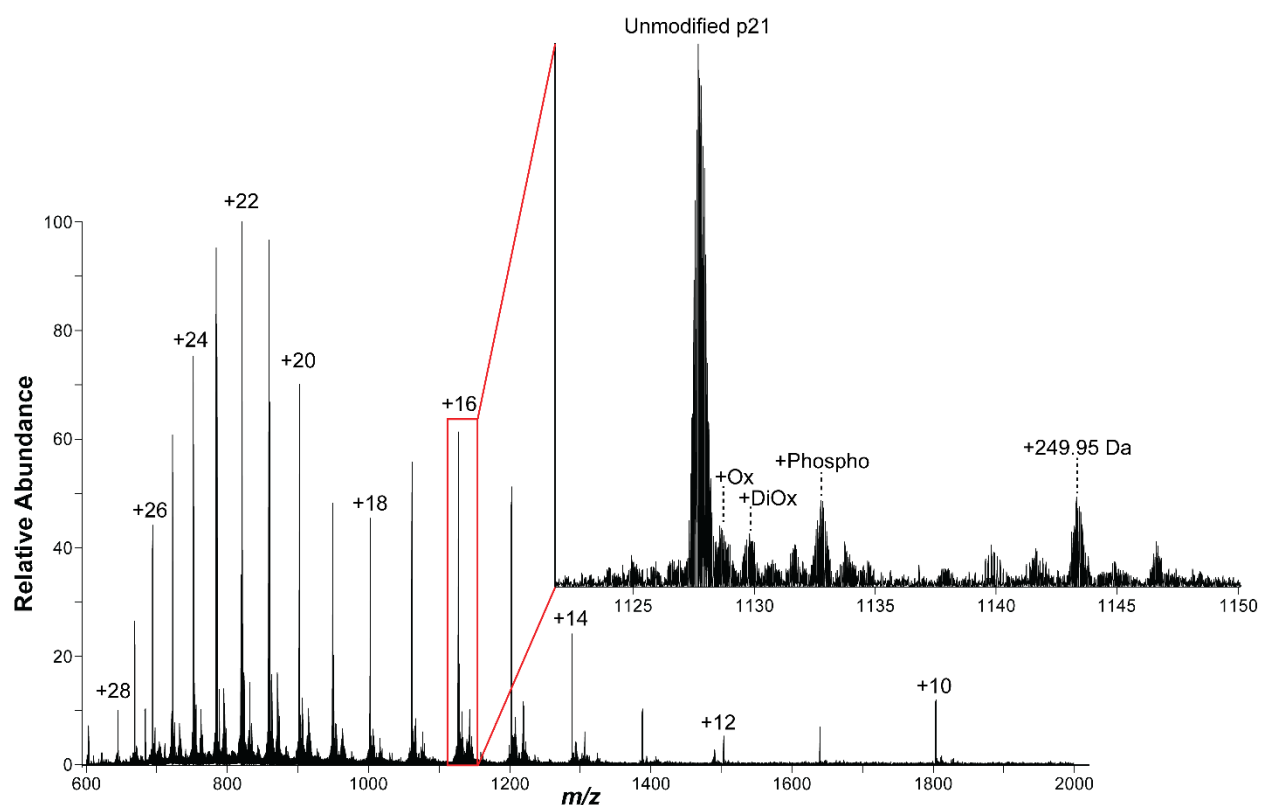
## Immunoprecipitation/Top-Down MS

Proteoforms Identified:

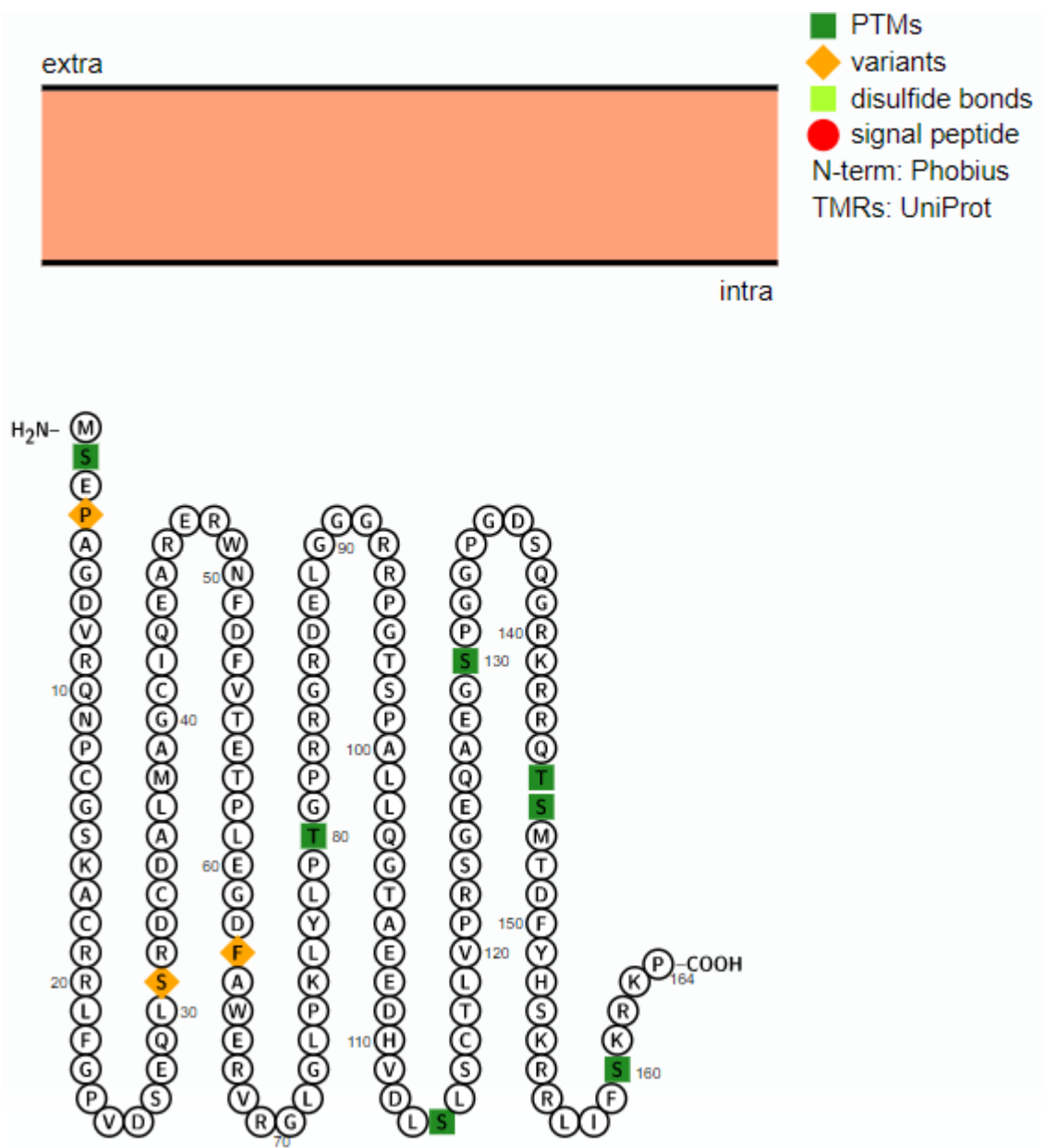
1: PFR465238, p21 Unmodified, 18,018.94 Da, [Acetyl]-  
SEPAGDVRQNPCGSKACRRLFGPVDSEQLSRDCDALMAGCIQEARERWNFDFVTETPLEGDF  
AWERVRLGLPKLYLPTGPRRGRDELGGRRPGTSPALLQGTAEEDHVDLSLSCTLVPRSGE  
QAEGLSPGGPGDSQGRKRRQTSMTDFYHSKRRLIFSKRKP

2: PFR599687, p21 Phosphorylated, 18,098.91 Da, [Acetyl]-  
SEPAGDVRQNPCGSKACRRLFGPVDSEQLSRDCDALMAGCIQEARERWNFDFVTETPLEGDF  
AWERVRLGLPKLYLPTGPRRGRDELGGRRPGTSPALLQGTAEEDHVDLS[Phospho]LCTL  
VPRSGEQAEGLSPGGPGDSQGRKRRQTSMTDFYHSKRRLIFSKRKP

Intact Mass Profile



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



## Supporting Information:

### Unmodified

**N** S E P A G D V R Q N P **C** G S K A **C** R R L F G P V D 25  
26 S E Q L S R D **C** D **A** **L** M A G **C** I Q E A R E R W N F 50  
51 **D** **F** **V** **T** E T P L E G D F A W E R V R G L G L P K L 75  
76 Y L P T G P R R G R D E L G G G R R P G T S P **A** **L** 100  
101 **L** **L** Q G T A E E D H V D L S **L** **S** **C** T L **V** **P** R S G E Q 125  
126 **A** **E** **G** **S** **P** **G** **G** **P** **G** D **S** Q G R K R R Q T S M T D **F** Y 150  
151 H S K R R L I F S K R K P **C**

### Phosphorylated

**N** S E P A G D V R Q N P **C** G S K A **C** R R L F G P V D 25  
26 S E Q L S R D **C** D **A** **L** M A G **C** I Q E A R E R W N F 50  
51 **D** **F** **V** **T** **E** **T** P L E G D F A W E R V R G L G L P K L 75  
76 Y L P T G P R R G R D E L G G G R R P G T S P A L 100  
101 **L** **L** Q G T A E E D H V D L **S** L **S** **C** T L **V** **P** R S G E Q 125  
126 A **E** **G** **S** **P** **G** **G** **P** **G** D **S** Q G R K R R Q T S M T D **F** Y 150  
151 H S K R R L I F S K R K P **C**