

Antibody Validation Report

aVR.PCNA.P12004.AB_2160343.v1.1 (November 3_2020)



A. Basic Target Information

Target Information

UniProt Accession Number: P12004

Target Name: Proliferating cell nuclear antigen (PCNA)

Antibody Information

RRID: AB_2160343

Antibody Name: PCNA (PC10) Mouse mAb antibody

Host Organism: Mouse

Clonality: Monoclonal

Vendor: Cell Signaling Technology

Catalog Number: 2586

Lot Number: 7

Recombinant (Y/N): No

Organ/Tissue used for validation: HeLa

HuBMAP Platform Used: IP-MS

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

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

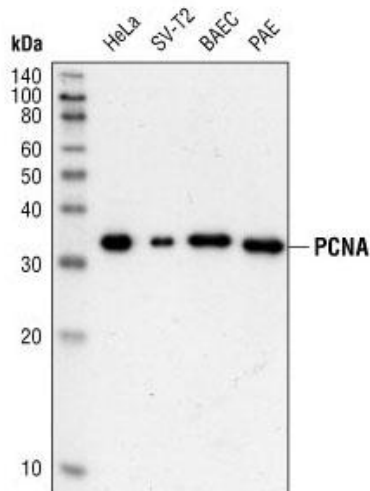
B. Validation Data

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

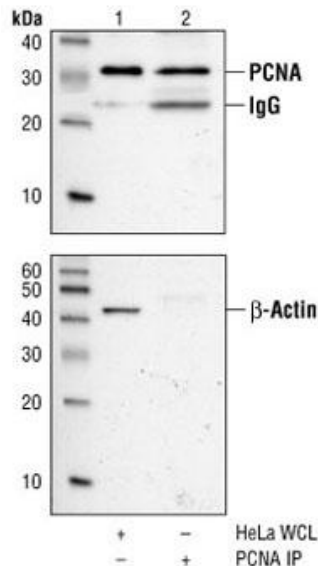
Date Accessed: 06/29/20

URL: <https://www.cellsignal.com/products/primary-antibodies/pcna-pc10-mouse-mab/2586>

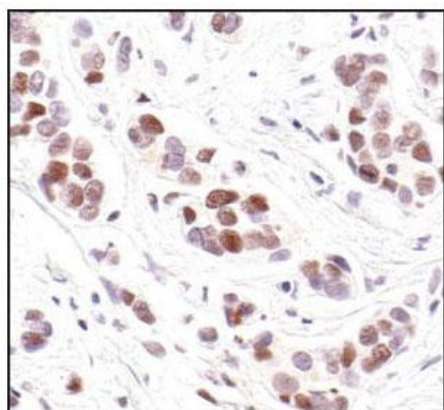
Western Blot



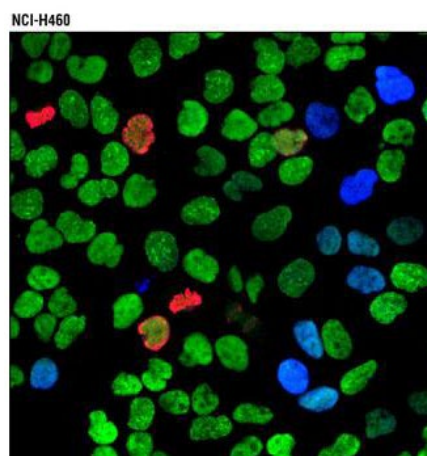
Immunoprecipitation and Western Blot



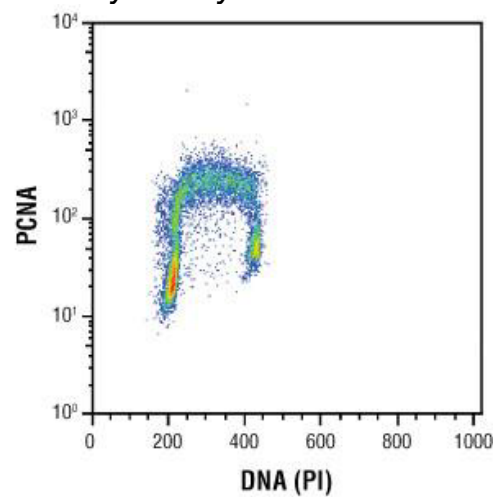
Immunohistochemistry



Immunofluorescence

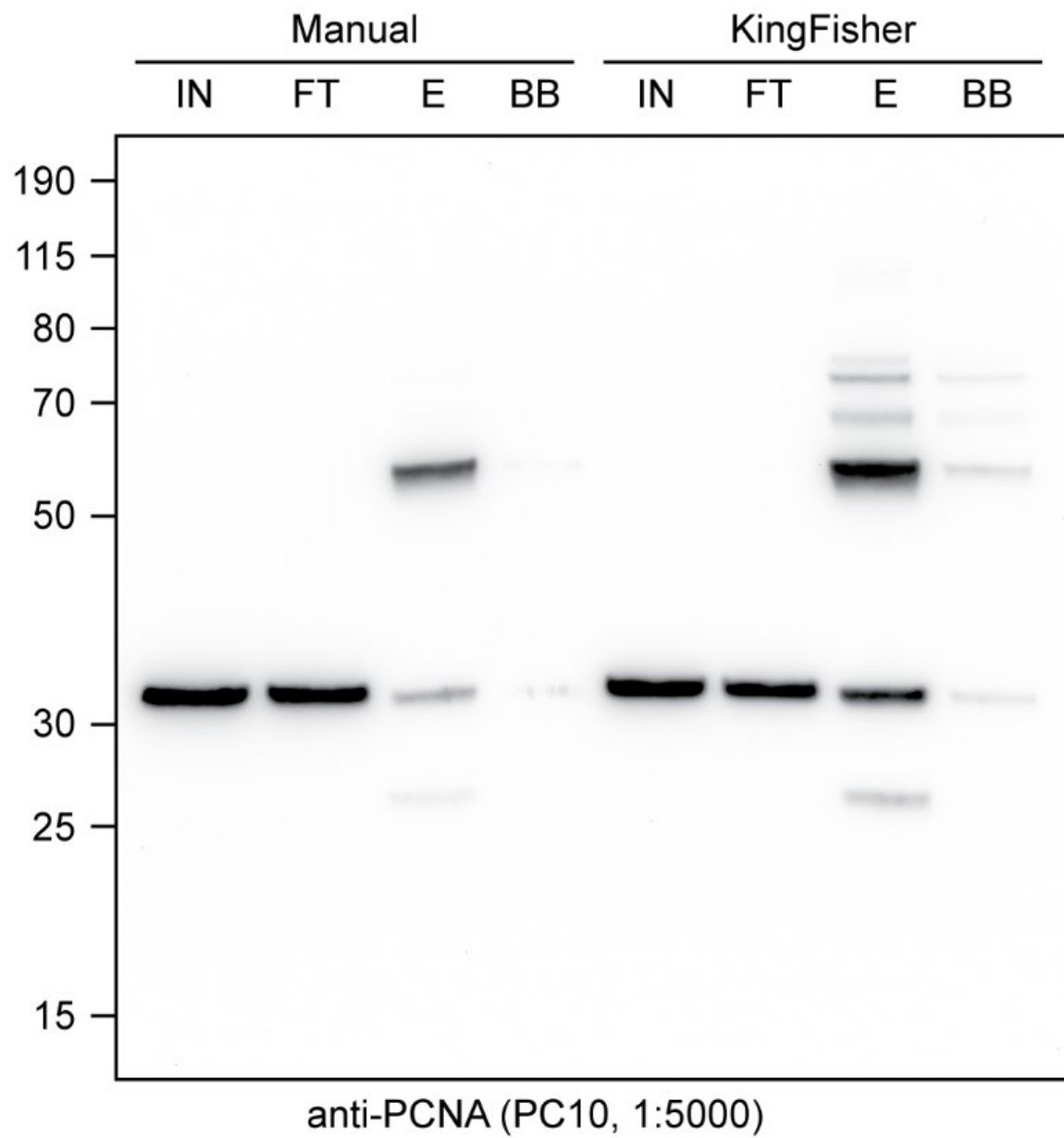


Flow Cytometry

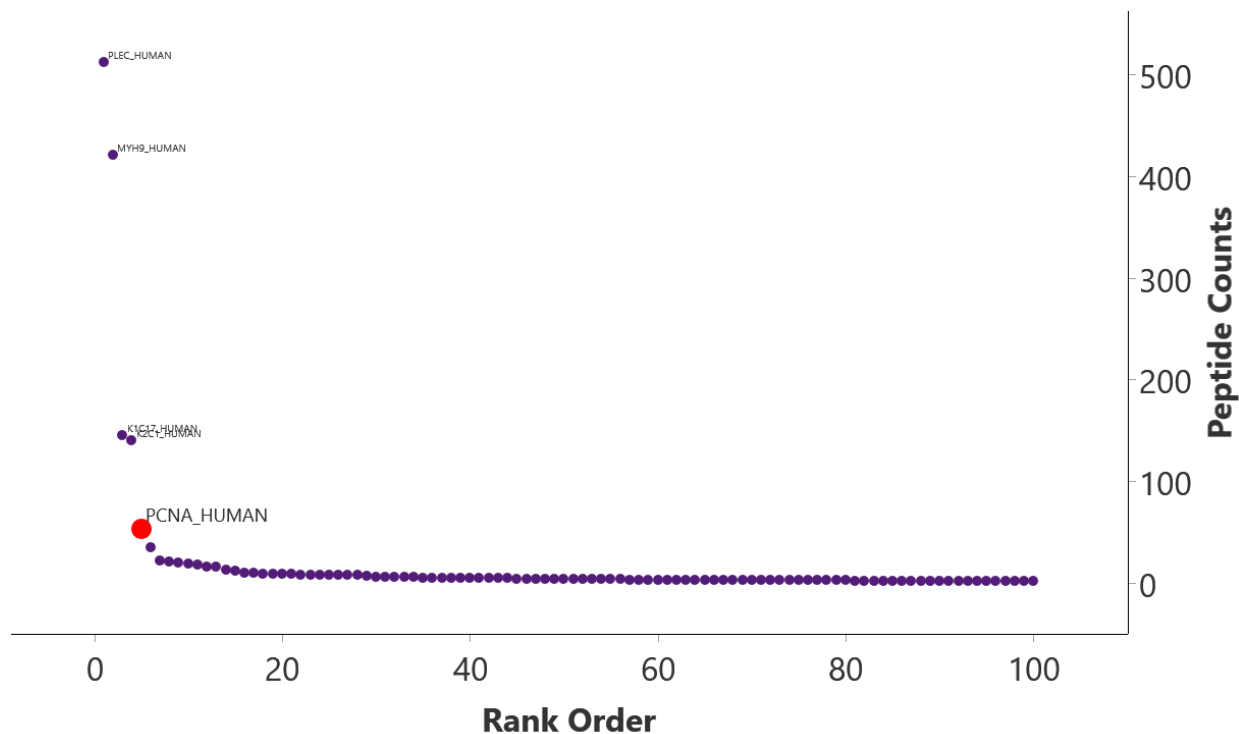


B.2. Laboratory Validation:

Immunoprecipitation/Western Blot



Immunoprecipitation/Bottom-Up MS



Immunoprecipitation/Top-Down MS

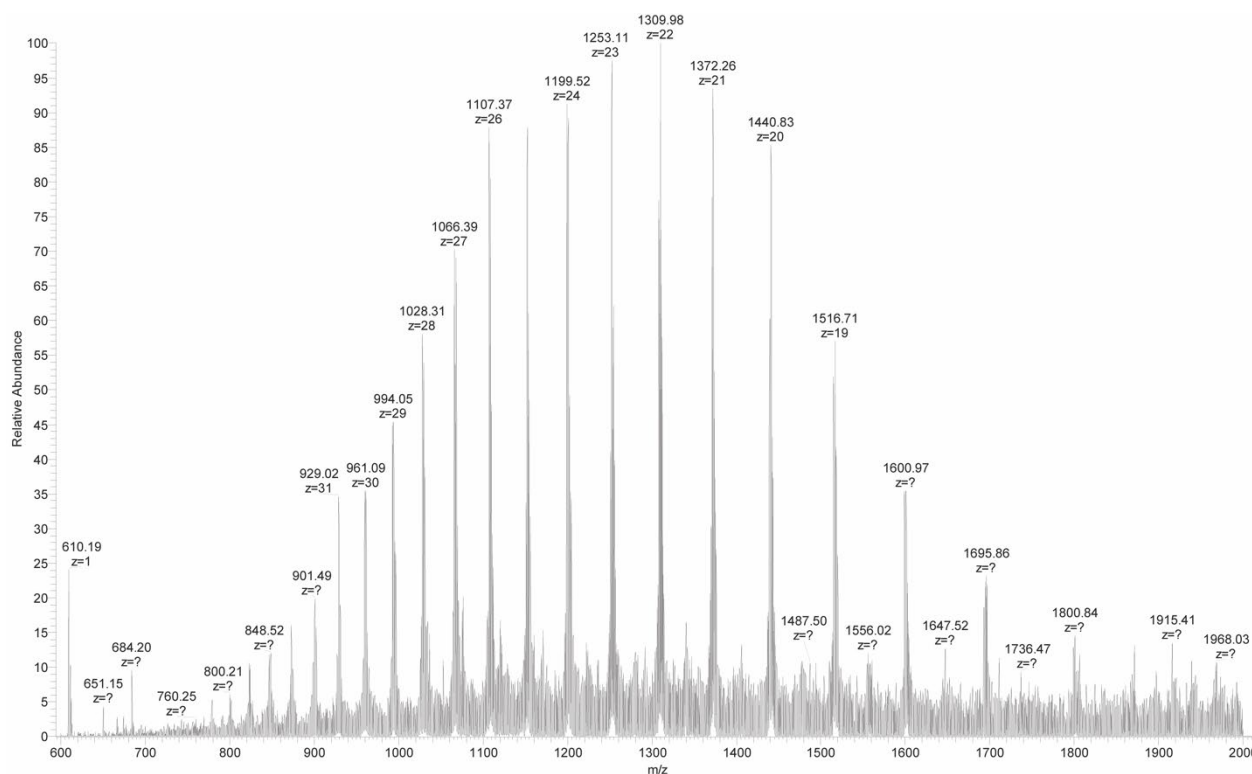
Proteoforms Identified:

1: PFR5430679, PCNA K-Ac/R-Cit, 28,793.29 Da,
MFEARLVQGSILKKVLEALKDLINEACWDISSSGVNLQSMDSHVSLVQLTLRSEGFD
TYR[MOD:00219]CDRNLAMGVNLTSMKILKCAGNEDIITLRAEDNADTLALVFEAPNQE
KVSDYEMKLMDL DVEQLGIPEQEYSCVVKMPSGEFARICRDLSHIGDAVVISCAKDG
VVF SASGELGNGNIKLSQTSNVDKEEEAVTIEMNEPVQLTFALRYLNFFTK[MOD:00064]ATP
LSSTVTLSMSADVPLVVEYKIADMGHLKYYLAPKIEDEEGS

2: PFR2382284, PCNA K-Ac2/Y-Ph, 28,914.28 Da,
MFEARLVQGSILKKVLEALKDLINEACWDISSSGVNLQSMDSHVSLVQLTLRSEGFD
TYRCDRNLAMGVNLTSMK[MOD:00064]ILK[MOD:00064]CAGNEDIITLRAEDNADTLAL
VFEAPNQEKVSDYEMKLMDL DVEQLGIPEQEYSCVVKMPSGEFARICRDLSHIGDAVVI
SCAKDGVKFSASGELGNGNIKLSQTSNVDKEEEAVTIEMNEPVQLTFALRY[MOD:0004
8]LNFFTKATPLSSTVTLSMSADVPLVVEYKIADMGHLKYYLAPKIEDEEGS

3: PFR623875, PCNA K-Ac1/Y-Ph, 28,872.27 Da,
MFEARLVQGSILKKVLEALKDLINEACWDISSSGVNLQSMDSHVSLVQLTLRSEGFD
TYRCDRNLAMGVNLTSMKILKCAGNEDIITLRAEDNADTLALVFEAPNQEKVSDYEMKL
MDLDVEQLGIPEQEYSCVVKMPSGEFARICRDLSHIGDAVVISCAKDGKFSASGELGN
GNIKLSQTSNVDKEEEAVTIEMNEPVQLTFALRY[MOD:00048]LNFFTKATPLSSTVTLS
MSADVPLVVEYKIADMGHLK[MOD:00064]YYLAPKIEDEEGS

Intact Mass Profile



IP-TDMS Graphical Fragment Map of PCNA

Lysine acetylation and arginine citrullination (PFR 5430679)

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

- Proteoform Characterization Score (PCS): 432
- P-score: 4.6×10^{-41}
- Fragment ions explained: 7%
- Backbone cleavages: 15%
- Proteoform Record Number (PFR): NA
- Mass of 3 Da between observed and theoretical unaccounted for based on fragmentation information

Lysine acetylation (2) and Phosphorylation PFR 2382284

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

- P-score: 2.7×10^{-35}
- Backbone cleavages: 9%
- Proteoform Record Number (PFR): 2382284

Lysine acetylation (1) and Phosphorylation: **PFR 623875**

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

- P-score: 3.2×10^{-32}
- Backbone cleavages: 8%
- Proteoform Record Number (PFR): 623875