

Antibody Validation Report

aVR.GATA3.P23771.AB_2819013.v1.0 (November 19_2020)



A. Basic Target Information

Target Information

UniProt Accession Number: P23771

Target Name: Trans-acting T-cell-specific transcription factor GATA-3

Antibody Information

RRID: AB_2819013

Antibody Name: Recombinant Anti-GATA3 antibody [EPR16651] - ChIP Grade

Host Organism: Rabbit

Clonality: Monoclonal

Vendor: Abcam

Catalog Number: ab199428

Lot Number: GR3266930-1

Recombinant (Y/N): Yes

Organ/Tissue used for validation: MCF7

HuBMAP Platform Used: IP-MS

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

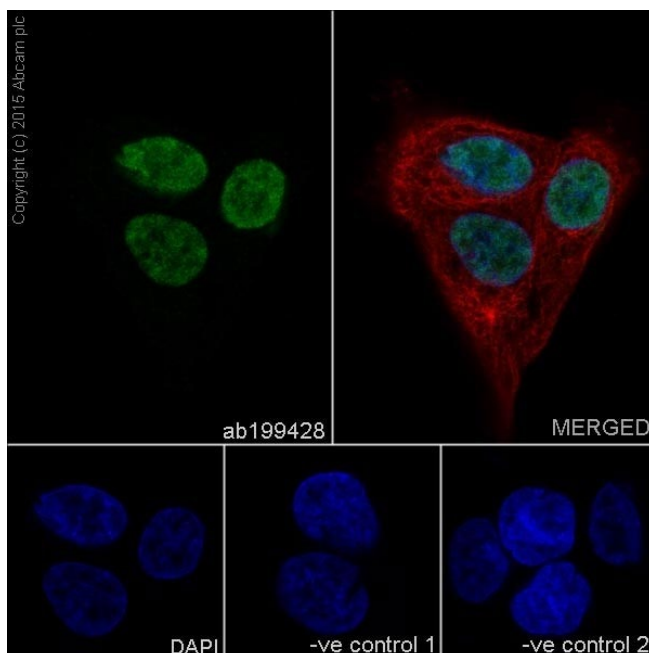
ORCID ID of submitter: 0000-0002-8815-3372

B. Validation Data

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

Date Accessed: 11/19/20

URL: <https://www.abcam.com/gata3-antibody-epr16651-chip-grade-ab199428.html>



Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized SH-SY5Y cells (Human neuroblastoma from bone marrow cells) labeling GATA3 with ab199428 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Nuclear staining on SH-SY5Y cell line is observed. The nuclear counterstain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1 - ab199428 at 1/250 dilution followed by ab150120 (Alexa Fluor® 594 Goat anti-Mouse secondary) at 1/500 dilution.

-ve control 2. - ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution followed by ab150077 (Alexa Fluor® 488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

B.2. Laboratory Validation:

Immunoprecipitation/Western Blot

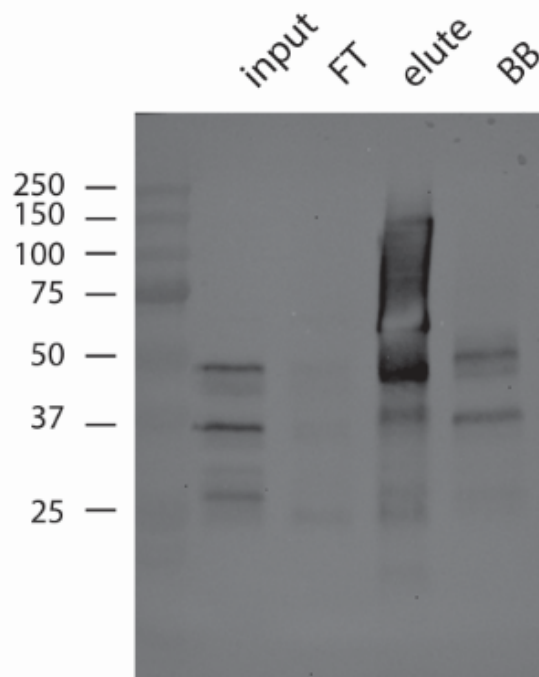
11/19/2020_Western blot: GATA-3 IP_MCF7

4-20% Protean gel

Cell signaling #5852 (D13C9) (rabbit monoclonal) 1:1000 on 4C

Secondary VeriBlot for IP (ab131366) 1:200 1 h RT

Amersham ECL WB detection reagent



IP: Abcam EPR16651/RIPA

IP conditions:

20ug Ab Abcam EPR16651 (32ul)

MCF7 (1 pellet from 5x15cm dishes)

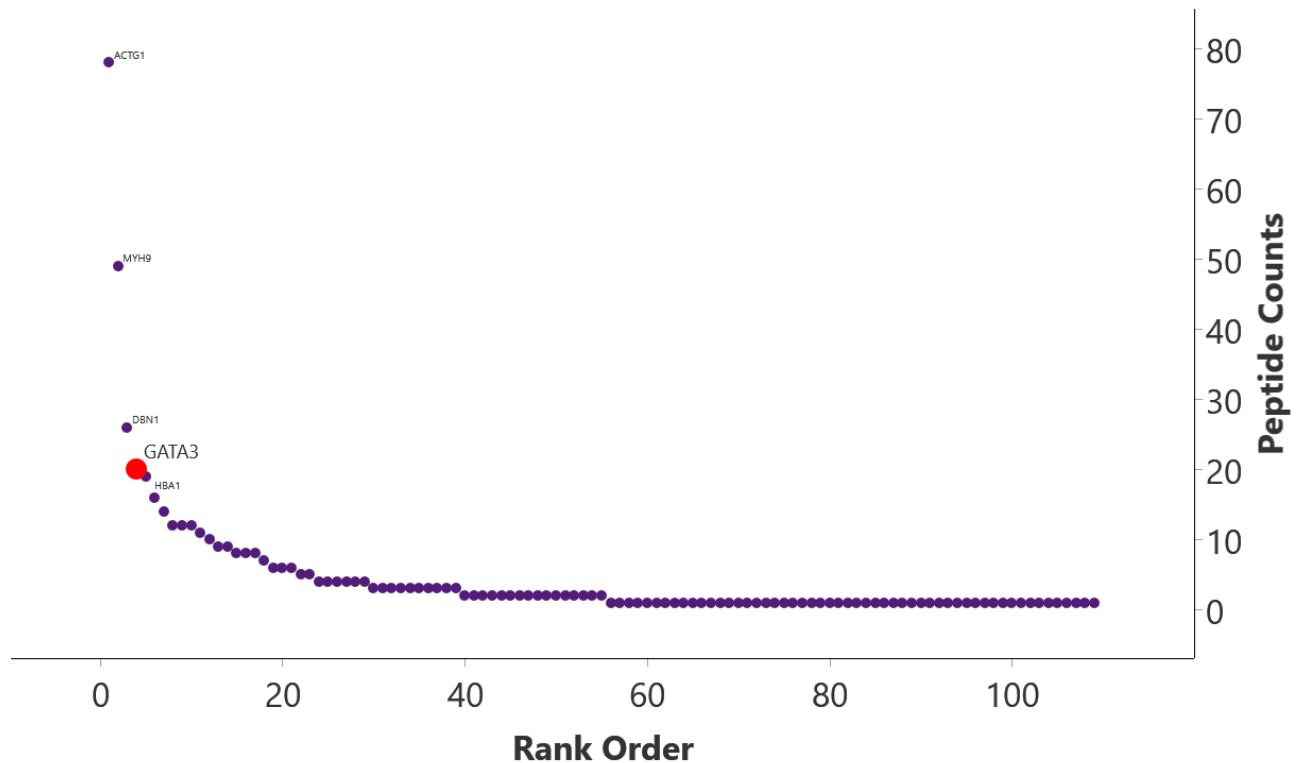
3 washes with 1ml Lysis buffer

5 washes with 1ml TBS1x

1 wash with Optima grade H2O

elution in 100ul 0.5% FA 10 min at 37C

Immunoprecipitation/Bottom-Up MS

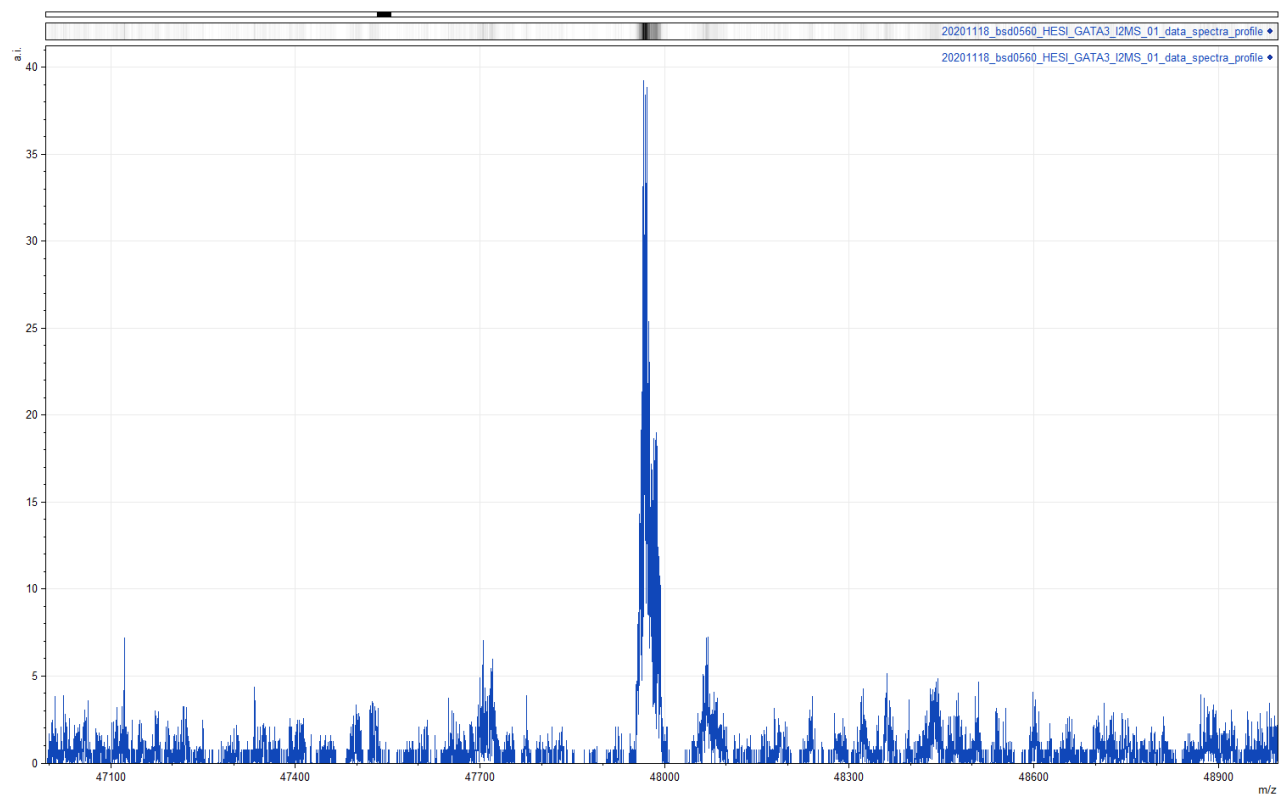


Immunoprecipitation/Top-Down MS

Proteoforms Identified:

1: PFR **TBD**, GATA-3 NAc-Ox, 47967.36 Da, [MOD:00425]?[MOD:00394]-
MEVTADQPRWVSHHHPAVLNGQHPDTHHPGLSHSYMDAAQYPLPEEVDVLFNIDGQGN
HVPPYYGNSVRATVQRYPPTHHGSQVCRPPLLHGSLPWLDGGKALGSHHTASPWNLS
FSKTSIHGSPGPLSVYPPASSSSLSGGHASPFLFTFPPTPPKDVSPDPSLSTPGSAGSA
RQDEKECLKYQVPLPDMSMKLESSHSRGSM TALGGASSSTHHPITTYPPYVPEYSSGLFP
PSSLLGGSPTGFGCKSRPKARSSTGRECVNCGATSTPLWRRDGTGHYLCNACGLYHKM
NGQNRPLIKPKRRLSAARRAGTSCANCQTTTTTLWRRNANGDPVCNACGLYYKLHNINR
PLTMKKEGIQTRNRKMSSKSKCKKVHDSLEDFPKNSSFNPAALSRHMSSLSHISPFSSHS
SHMLTTPTPMHPPSSLSFGPHHPSSMVTAMG

Intact Mass Profile



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

No Protter Data Available, intracellular protein.