

## **Antibody Validation Report**

aVR.TNF.P01375.AB\_2889388.v1.0 (March 25\_2021)



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

#### **Target Information**

**UniProt Accession Number:** P01375

**Target Name:** Tumor necrosis factor

#### **Antibody Information**

**RRID:** AB\_2889388

**Antibody Name:** Recombinant Anti-TNF alpha antibody [EPR19147]

**Host Organism:** Rabbit

**Clonality:** Monoclonal

**Vendor:** AbCam

**Catalog Number:** ab183218

**Lot Number:** GR3341243-4

**Recombinant (Y/N):** Yes

**Organ/Tissue used for validation:** THP-1

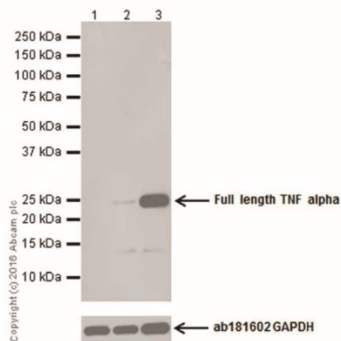
**HuBMAP Platform Used:** IP-MS

**Protocols.io doi for Validation Protocol:** 10.17504/protocols.io.btzqnp5w

**ORCID ID of submitter:** 0000-0002-6095-2797

### **B. Validation Data**

#### **B.1. Vendor Validation: IP, WB, IF**



Anti-TNF alpha antibody [EPR19147]

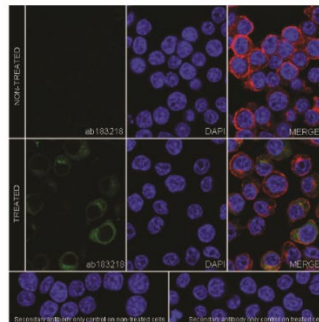
(ab183218), 1/2000 dilution

Lane 1 : TPA pretreated THP-1

Lane 2 : TPA pretreated THP-1 treated with 100 ng/ml LPS for 7 hours

Lane 3 : TPA pretreated THP-1 treated with 100 ng/ml LPS for 4 hours and 1 µg/ml BFA for 3 hours

Secondary: Goat Anti-Rabbit IgG H&L (HRP) (ab97051), 1/100000 dilution



RAW264.7 treated with 100ng/ml LPS for 7 h and 1µg/ml BFA for the last 3h

Primary Antibody: anti- TNF alpha (Abcam\_ ab183218), 1/5000 dilution (0.09 µg/ml).

Secondary Antibody: Goat anti rabbit IgG (Alexa Fluor® 488, ab150077), 1/1000 dilution (2 µg/ml)

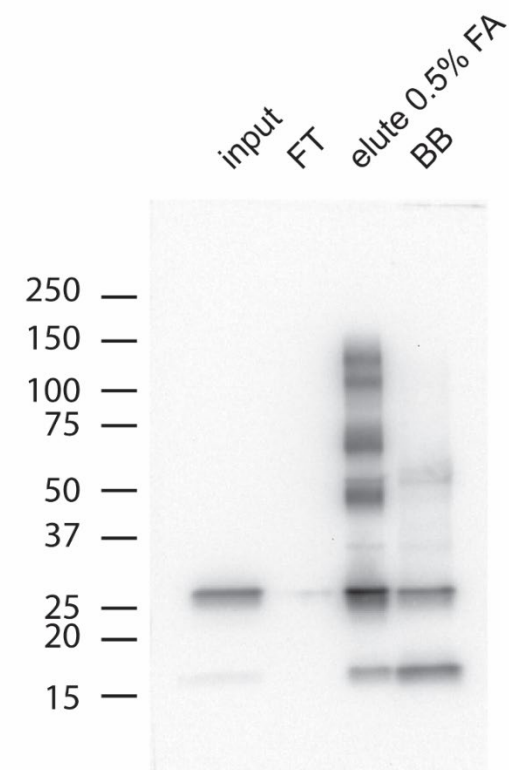
Date Accessed: 03/25/21

URL: <https://www.abcam.com/tnf-alpha-antibody-epr19147-ab183218.html>

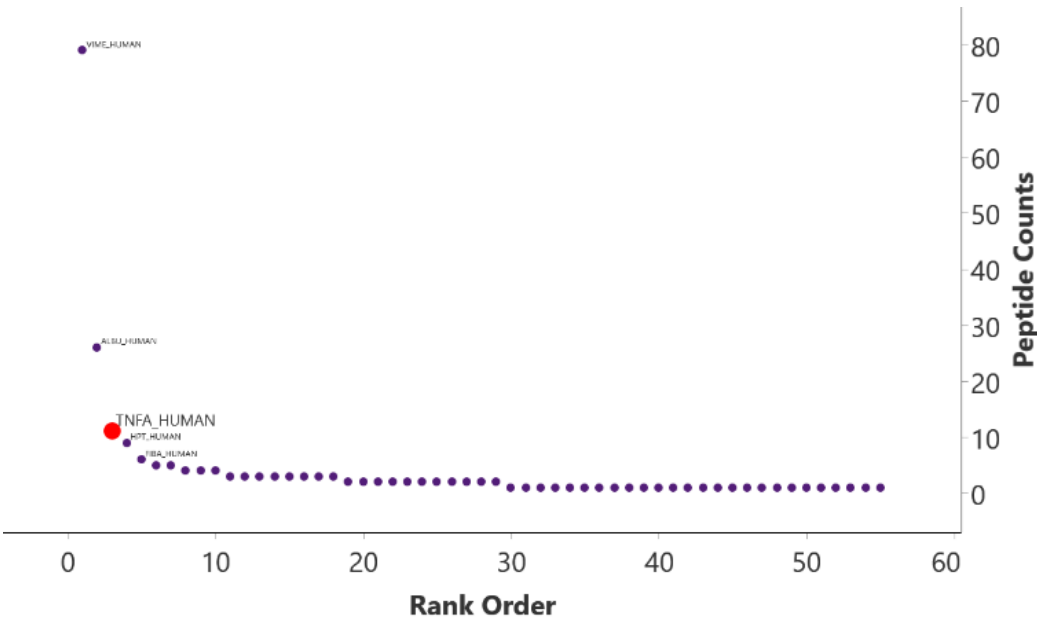
**B.2. Laboratory Validation:**

**Immunoprecipitation/Western Blot**

Primary anti-TNF (Abcam \_ab183218\_ RRID: 2889388)1:1000 on 4C  
Secondary VeriBlot for IP Detection Reagent (HRP) (ab131366) 1/300 1 h RT



**Immunoprecipitation/Bottom-Up MS**



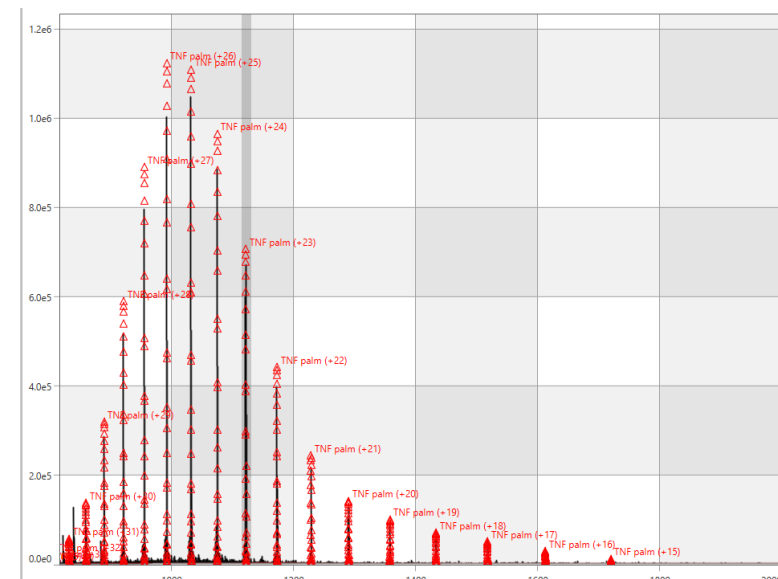
## Immunoprecipitation/Top-Down MS

Proteoforms Identified:

1: PFR00005931241, Palmitoylated TNF alpha, 25,775.55 Da

[MOD:00394]-

STESMIRDVELAEALPKKTGGPQGSRRRC[MOD:00440]LFLSLFSLIVAGATTLFCLLHFGVIGPQREEFPRDL  
SLISPLAQAVRSSRTPSDKPVAVHVVANPQAEGLQWLNRRANALLANGVELRDNQLVVPSEGLYLIYSQVLF  
KGQGC[MOD:00798]PSTHVLLTHTISRIVSYQTKVNLLSAIKSP[MOD:00798]CQRETPEGAEAKPWYEPIY  
LGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIL



<b>N</b> <b>S</b> T E S M I R D V E L A E E A L P K K T G G P Q G 25		<b>Precursor Mass</b>	
26 S R R C L F L S L F S F L I V A G A T T L F L L 50		Type: Monoisotopic	
51 H F G V I G P Q R E E F P R D L S L I S P L A Q A 75		Observed: 25,775.89	
76 V R S S S R T P S D K P V A H V V A N P Q A E G Q 100		Theoretical: 25,775.55	
101 L Q W L N R R A N A L L A N G V E L R D N Q L V V 125		Mass Diff. (Da): 0.346	
126 P S E G L Y L I Y S Q V L F K G Q G C P S T H V L 150		Mass Diff. (ppm): 13.42	
151 L T H T I S R I A V S Y Q T K V N L L S A I K S P 175		<b>Scores</b>	
176 C Q R E T P E G A E A K P W Y E P I Y L G G V F Q 200		PCS: 126.11	
201 L E K G D R L S A E I N R P D Y L D F A E S G Q V 225		P-Score: 3.7e-16	
226 Y F G I I A L C		% Fragments Expl... 13%	
		% Residue Cleava... 6%	
		<b>Modification (S1)</b>	
		<input checked="" type="checkbox"/> No Modification	
		<input type="checkbox"/> Custom <input type="text"/>	
		Common	
		<input checked="" type="checkbox"/> Phosphorylation	
		Uncommon	
		<input checked="" type="checkbox"/> Monomethylation	
		<input checked="" type="checkbox"/> Acetylation	

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

