

### VCP Antibody

Purified Mouse Monoclonal Antibody (Mab) Catalog # AM8494b

### Specification

# VCP Antibody - Product Information

Application Primary Accession Reactivity Host Clonality Isotype WB, IHC-P, FC, IF,E <u>P55072</u> Human, Mouse, Rat Mouse monoclonal IgG1,ĸ

### VCP Antibody - Additional Information

Gene ID 7415

**Other Names** Transitional endoplasmic reticulum ATPase, TER ATPase, 15S Mg(2+)-ATPase p97 subunit, Valosin-containing protein, VCP, VCP

**Target/Specificity** 

This VCP antibody is generated from a mouse immunized with a recombinant protein of human TERA.

Dilution WB~~1:4000 IHC-P~~1:25 FC~~1:25 IF~~1:25 E~~Use at an assay dependent concentration.

Format

Purified monoclonal antibody supplied in PBS with 0.09% (W/V) sodium azide. This antibody is purified through a protein G column, followed by dialysis against PBS.

Storage

Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.

**Precautions** VCP Antibody is for research use only and not for use in diagnostic or therapeutic procedures.

# VCP Antibody - Protein Information

Name VCP

Function Necessary for the fragmentation of Golgi stacks during mitosis and for their reassembly



after mitosis. Involved in the formation of the transitional endoplasmic reticulum (tER). The transfer of membranes from the endoplasmic reticulum to the Golgi apparatus occurs via 50-70 nm transition vesicles which derive from part-rough, part-smooth transitional elements of the endoplasmic reticulum (tER). Vesicle budding from the tER is an ATP-dependent process. The ternary complex containing UFD1, VCP and NPLOC4 binds ubiguitinated proteins and is necessary for the export of misfolded proteins from the ER to the cytoplasm, where they are degraded by the proteasome. The NPLOC4- UFD1-VCP complex regulates spindle disassembly at the end of mitosis and is necessary for the formation of a closed nuclear envelope. Regulates E3 ubiquitin-protein ligase activity of RNF19A. Component of the VCP/p97-AMFR/gp78 complex that participates in the final step of the sterol-mediated ubiquitination and endoplasmic reticulum-associated degradation (ERAD) of HMGCR. Mediates the endoplasmic reticulum- associated degradation of CHRNA3 in cortical neurons as part of the STUB1-VCP-UBXN2A complex (PubMed: 26265139). Involved in endoplasmic reticulum stress-induced pre-emptive quality control, a mechanism that selectively attenuates the translocation of newly synthesized proteins into the endoplasmic reticulum and reroutes them to the cytosol for proteasomal degradation (PubMed: 26565908). Involved in clearance process by mediating G3BP1 extraction from stress granules (PubMed: 29804830, PubMed: 34739333). Also involved in DNA damage response: recruited to double-strand breaks (DSBs) sites in a RNF8- and RNF168-dependent manner and promotes the recruitment of TP53BP1 at DNA damage sites (PubMed:22020440, PubMed:22120668). Recruited to stalled replication forks by SPRTN: may act by mediating extraction of DNA polymerase eta (POLH) to prevent excessive translesion DNA synthesis and limit the incidence of mutations induced by DNA damage (PubMed:23042605, PubMed:23042607). Together with SPRTN metalloprotease, involved in the repair of covalent DNA-protein cross- links (DPCs) during DNA synthesis (PubMed: 32152270). Involved in interstrand cross-link repair in response to replication stress by mediating unloading of the ubiquitinated CMG helicase complex (By similarity). Mediates extraction of PARP1 trapped to chromatin: recognizes and binds ubiquitinated PARP1 and promotes its removal (PubMed: 35013556). Required for cytoplasmic retrotranslocation of stressed/damaged mitochondrial outer-membrane proteins and their subsequent proteasomal degradation (PubMed: 16186510, PubMed: 21118995). Essential for the maturation of ubiquitin-containing autophagosomes and the clearance of ubiquitinated protein by autophagy (PubMed: 20104022, PubMed:27753622). Acts as a negative regulator of type I interferon production by interacting with RIGI: interaction takes place when RIGI is ubiquitinated via 'Lys-63'-linked ubiquitin on its CARD domains, leading to recruit RNF125 and promote ubiguitination and degradation of RIGI (PubMed: <u>26471729</u>). May play a role in the ubiguitin-dependent sorting of membrane proteins to lysosomes where they undergo degradation (PubMed:<u>21822278</u>). May more particularly play a role in caveolins sorting in cells (PubMed:21822278, PubMed:23335559). By controlling the steady-state expression of the IGF1R receptor, indirectly regulates the insulin-like growth factor receptor signaling pathway (PubMed: 26692333).

#### **Cellular Location**

Cytoplasm, cytosol. Endoplasmic reticulum. Nucleus. Cytoplasm, Stress granule. Note=Present in the neuronal hyaline inclusion bodies specifically found in motor neurons from amyotrophic lateral sclerosis patients (PubMed:15456787). Present in the Lewy bodies specifically found in neurons from Parkinson disease patients (PubMed:15456787). Recruited to the cytoplasmic surface of the endoplasmic reticulum via interaction with AMFR/gp78 (PubMed:16168377) Following DNA double-strand breaks, recruited to the sites of damage (PubMed:22120668). Recruited to stalled replication forks via interaction with SPRTN (PubMed:23042605). Recruited to damaged lysosomes decorated with K48-linked ubiquitin chains (PubMed:27753622) Colocalizes with TIA1, ZFAND1 and G3BP1 in cytoplasmic stress granules (SGs) in response to arsenite-induced stress treatment (PubMed:29804830).

# VCP Antibody - Protocols

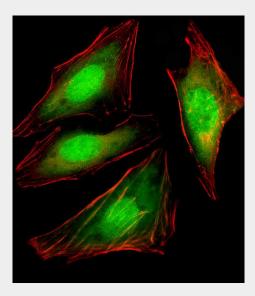
Provided below are standard protocols that you may find useful for product applications.

<u>Western Blot</u>

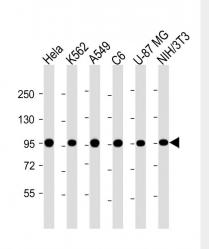


- <u>Blocking Peptides</u>
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- <u>Cell Culture</u>

#### VCP Antibody - Images



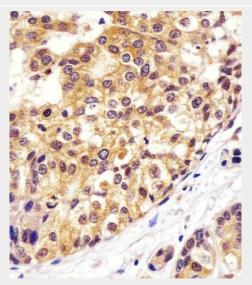
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervical epithelial adenocarcinoma cell line) cells labeling TERA with AM8494b at 1/25 dilution, followed by Dylight® 488-conjugated goat anti-mouse IgG (NA166821) secondary antibody at 1/200 dilution (green). Immunofluorescence image showing cytoplasm and nucleus staining on HeLa cell line. Cytoplasmic actin is detected with Dylight® 554 Phalloidin (PD18466410) at 1/100 dilution (red).



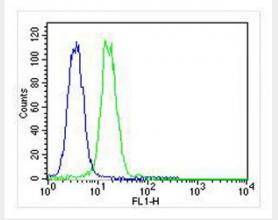
All lanes : Anti-VCP Antibody at 1:4000 dilution Lane 1: Hela whole cell lysate Lane 2: K562 whole cell lysate Lane 3: A549 whole cell lysate Lane 4: C6 whole cell lysate Lane 5: U-87 MG whole cell lysate Lane 6: NIH/3T3 whole cell lysate Lysates/proteins at 20  $\mu$ g per lane. Secondary Goat Anti-mouse IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 89 kDa



Blocking/Dilution buffer: 5% NFDM/TBST.



AM8494b staining VCP in human breast carcinoma sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 3% BSA for 0. 5 hour at room temperature; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody (1/25) for 1 hours at 37°C. A undiluted biotinylated goat polyvalent antibody was used as the secondary antibody.



Overlay histogram showing K562 cells stained with AM8494b (green line). The cells were fixed with 2% paraformaldehyde (10 min) and then permeabilized with 90% methanol for 10 min. The cells were then icubated in 2% bovine serum albumin to block non-specific protein-protein interactions followed by the antibody (AM8494b, 1:25 dilution) for 60 min at 37°C. The secondary Goat-Anti-Mouse antibody used was lgG, **DyLight**® 488 Conjugated Highly Cross-Adsorbed(NA168821)) at 1/400 dilution for 40 min at 37°C. Isotype control antibody (blue line) was mouse  $IgG1 (1\mu g/1x10^6 cells)$  used under the same conditions. Acquisition of >10, 000 events was performed.

# VCP Antibody - Background

Necessary for the fragmentation of Golgi stacks during mitosis and for their reassembly after mitosis. Involved in the formation of the transitional endoplasmic reticulum (tER). The transfer of membranes from the endoplasmic reticulum to the Golgi apparatus occurs via 50-70 nm transition vesicles which derive from part-rough, part-smooth transitional elements of the endoplasmic reticulum (tER). Vesicle budding from the tER is an ATP-dependent process. The ternary complex containing UFD1L, VCP and NPLOC4 binds ubiquitinated proteins and is necessary for the export of misfolded proteins from the ER to the cytoplasm, where they are degraded by the proteasome. The



NPLOC4-UFD1L-VCP complex regulates spindle disassembly at the end of mitosis and is necessary for the formation of a closed nuclear envelope. Regulates E3 ubiquitin-protein ligase activity of RNF19A. Component of the VCP/p97-AMFR/gp78 complex that participates in the final step of the sterol-mediated ubiquitination and endoplasmic reticulum-associated degradation (ERAD) of HMGCR. Also involved in DNA damage response: recruited to double-strand breaks (DSBs) sites in a RNF8- and RNF168-dependent manner and promotes the recruitment of TP53BP1 at DNA damage sites. Recruited to stalled replication forks by SPRTN: may act by mediating extraction of DNA polymerase eta (POLH) to prevent excessive translesion DNA synthesis and limit the incidence of mutations induced by DNA damage. Required for cytoplasmic retrotranslocation of stressed/damaged mitochondrial outer-membrane proteins and their subsequent proteasomal degradation.

### VCP Antibody - References

Lamerdin J.E., et al. Submitted (MAR-1998) to the EMBL/GenBank/DDBJ databases. Hu R.-M., et al. Proc. Natl. Acad. Sci. U.S.A. 97:9543-9548(2000). Ota T., et al.Nat. Genet. 36:40-45(2004). Humphray S.J., et al.Nature 429:369-374(2004). Mural R.J., et al.Submitted (SEP-2005) to the EMBL/GenBank/DDBJ databases.