

Anti-VCP Antibody

Catalog Number: PA2137

About VCP

Valosin-containing protein also called CDC48 is an enzyme that in humans is encoded by the VCP gene. It is a member of the AAA+ (ATPase associated with various activities) protein family. The VCP gene maps to chromosome 9p13.3. It is necessary for the fragmentation of Golgi stacks during mitosis and for their reassembly after mitosis. It is involved in the formation of the transitional endoplasmic reticulum. This gene plays a role in vesicle transport and fusion, 26S proteasome function, and assembly of peroxisomes. It 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.

Overview

Product Name	Anti-VCP Antibody
Reactive Species	Human, Monkey, Mouse, Rat
Description	Boster Bio Anti-VCP Antibody catalog # PA2137. Tested in Flow Cytometry, IF, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, IHC-F, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg Thimerosal, 0.01mg NaN3.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P55072

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence at the C-terminus of human VCP, identical to the related rat and mouse sequences.
Predicted Reactive Species	Hamster
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P), IHC(F) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG
Form	Lyophilized

Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml, Human, Monkey, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, Mouse, Rat, By Heat Immunohistochemistry (Frozen Section), 0.5-1ug/ml, Rat Immunocytochemistry , 0.5- 1ug/ml, Human Immunocytochemistry/Immunofluorescence, 5ug/ml, Human Flow Cytometry, 1-3ug/1x10⁶ cells, Human

Anti-VCP Antibody (PA2137) Images

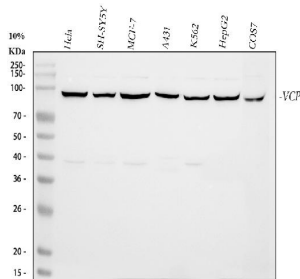


Figure 1. Western blot analysis of VCP using anti-VCP antibody (PA2137).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,
Lane 2: human SH-SY5Y whole cell lysates,
Lane 3: human MCF-7 whole cell lysates,
Lane 4: human A431 whole cell lysates,
Lane 5: human K562 whole cell lysates,
Lane 6: human HepG2 whole cell lysates,
Lane 7: monkey COS-7 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-VCP antigen affinity purified polyclonal antibody (Catalog # PA2137) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for VCP at approximately 97 kDa. The expected band size for VCP is at 89 kDa.

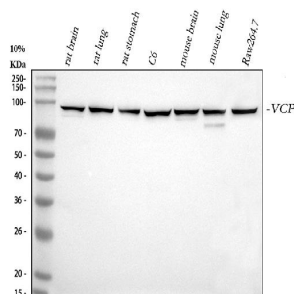


Figure 2. Western blot analysis of VCP using anti-VCP antibody (PA2137).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: rat brain tissue lysates,
Lane 2: rat lung tissue lysates,
Lane 3: rat stomach tissue lysates,
Lane 4: rat C6 whole cell lysates,
Lane 5: mouse brain tissue lysates,
Lane 6: mouse lung tissue lysates,
Lane 7: mouse RAW264.7 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-VCP antigen affinity purified polyclonal antibody (Catalog # PA2137) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for VCP at approximately 97 kDa. The expected band size for VCP is at 89 kDa.

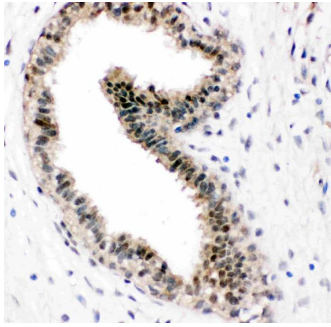


Figure 3. IHC analysis of VCP using anti-VCP antibody (PA2137). VCP was detected in paraffin-embedded section of Human Mammary Cancer tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VCP Antibody (PA2137) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

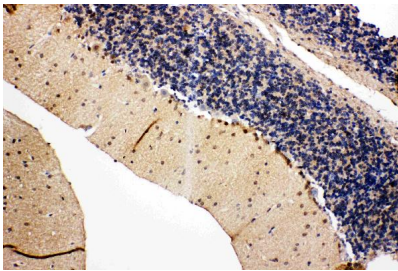


Figure 4. IHC analysis of VCP using anti-VCP antibody (PA2137). VCP was detected in paraffin-embedded section of Rat Cerebellum tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VCP Antibody (PA2137) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

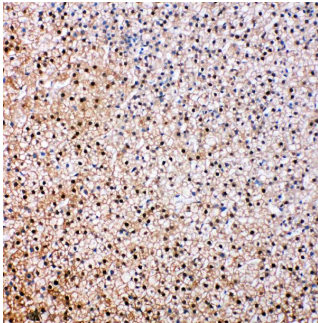


Figure 5. IHC analysis of VCP using anti-VCP antibody (PA2137). VCP was detected in paraffin-embedded section of Rat Epinephros tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VCP Antibody (PA2137) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

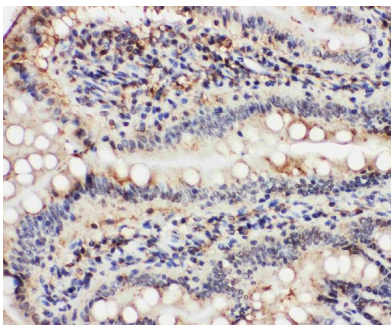
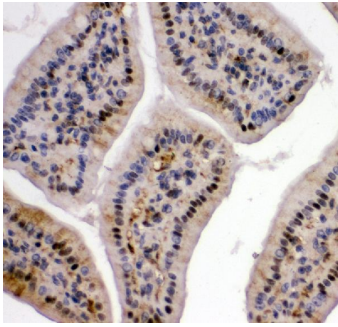


Figure 6. IHC analysis of VCP using anti-VCP antibody (PA2137). VCP was detected in paraffin-embedded section of Rat Intestine tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-VCP Antibody (PA2137) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

Figure 7. IHC analysis of VCP using anti-VCP antibody (PA2137).

VCP was detected in paraffin-embedded section of mouse intestine tissue. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml



rabbit anti-VCP Antibody (PA2137) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

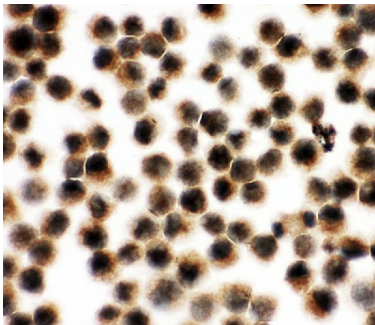


Figure 8. ICC analysis of VCP using anti-VCP antibody (PA2137).

VCP was detected in immunocytochemical section of K562 cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 1ug/ml rabbit anti-VCP Antibody (PA2137) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

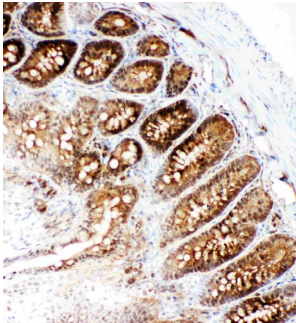


Figure 9. IHC analysis of VCP using anti-VCP antibody (PA2137).

VCP was detected in a frozen section of Rat Intestine tissue. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 ug/ml rabbit anti-VCP Antibody (PA2137) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) (Catalog # SA1022) with DAB as the chromogen.

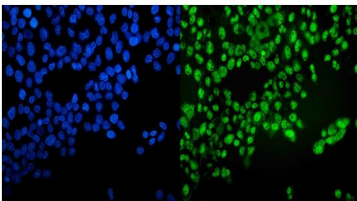
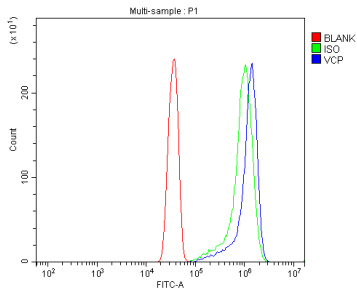


Figure 10. IF analysis of VCP using anti- VCP antibody (PA2137).

VCP was detected in immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5ug/mL rabbit anti-VCP Antibody (PA2137) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Figure 11. Flow Cytometry analysis of HELA cells using anti-VCP antibody (PA2137).

Overlay histogram showing HELA cells stained with PA2137 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-VCP Antibody (PA2137, 1ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10ug/1x10⁶



cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 μ g/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

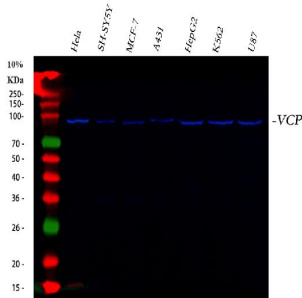


Figure 12. Western blot analysis of VCP using anti-VCP antibody (PA2137).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 μ g of sample under reducing conditions.

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Lane 5: human HepG2 whole cell lysates,
Lane 6: human K562 whole cell lysates,
Lane 7: human U87 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-VCP antigen affinity purified polyclonal antibody (Catalog # PA2137) at 0.5 μ g/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-DyLight 488 secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. A specific band was detected for VCP at approximately 97 kDa. The expected band size for VCP is at 89 kDa.

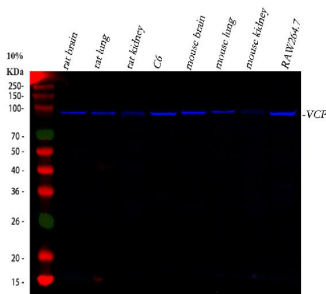


Figure 13. Western blot analysis of VCP using anti-VCP antibody (PA2137).

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