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Catalog #	Aliquot Size
V116-310G -05	5 µg
V116-310G -10	10 µg

VCP, Active

Recombinant full-length protein expressed in Sf9 cells

Catalog # V116-310G

Lot # B2097-7

Product Description

Recombinant full-length human VCP was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is NM_007126.

Gene Aliases

ALS14; CMT2Y; HEL-220; HEL-S-70; IBMPFD; IBMPFD1; p97; TERA

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

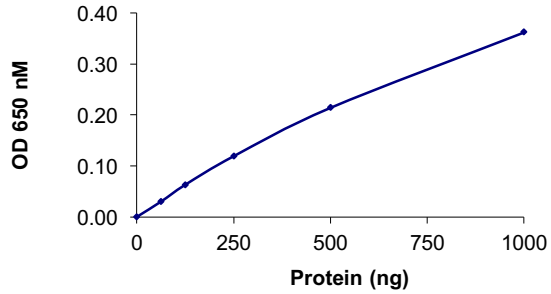
Scientific Background

VCP (valosin-containing protein or transitional endoplasmic reticulum ATPase) is a hexameric ATPase. Like many other members of the AAA (ATPases associated with diverse cellular activities) family, it uses the energy of ATP hydrolysis to structurally remodel client molecules. VCP facilitates the degradation of polyubiquitylated substrates in the proteasome. VCP/p97 has also been linked to various membrane trafficking processes, including Golgi reassembly following mitosis.

References

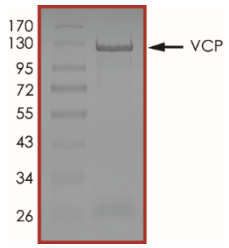
1. Meyer H, et al: The VCP/p97 system at a glance: connecting cellular function to disease pathogenesis. J Cell Sci. 2014 Sep 15;127(Pt 18):3877-83.
2. Erzberger JP, et al: Evolutionary relationships and structural mechanisms of AAA+ proteins. Annu Rev Biophys Biomol Struct. 2006;35:93-114.
3. Meyer HH. Golgi reassembly after mitosis: the AAA family meets the ubiquitin family. Biochim Biophys Acta. 2005 Jun 30;1744(2):108-19. Review. Erratum in: Biochim Biophys Acta. 2005 Dec 15;1746(2):169.

Specific Activity



The specific activity of VCP was determined to be **140 nmol /min/mg** as per activity assay protocol.

Purity



The purity of VCP was determined to be **>85%** by densitometry, approx. MW **120 kDa**.

VCP, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	V116-310G
Specific Activity	140 nmol/min/mg
Lot #	*B2097-7*
Purity	>85%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active ATPase (Catalog #: V116-310G)

Active VCP (0.1μg/μl) diluted with Kinase Dilution Buffer IX (Catalog #: K29-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active VCP for optimal results).

Kinase Dilution Buffer IX (Catalog #: K29-09)

Kinase Assay Buffer III (Catalog #: K03-09) diluted at a 1:4 ratio (5X dilution) with freshly prepared solution containing 50 μM DTT.

Kinase Assay Buffer III (Catalog #: K03-09)

Buffer components: 200mM Tris-HCl, pH7.4, 100mM MgCl2 and 0.5mg/ml BSA.

ATP (Catalog #: A50-09)

ATP stock solution at a concentration of 10mM. Dilute at a 1:1 ratio in Kinase Dilution Buffer IX prior to use.

Detection Solution

BIOMOL GREEN reagent phosphatase detection kit (BioMol Catalog #: AK-111).

Assay Protocol

Step 1. Prepare a fresh batch of Kinase Dilution Buffer and keep on ice.

Step 2. Prepare phosphate standard curve following the instruction of BIOMOL GREEN reagent phosphatase detection kit. Briefly, prepare 1:1 serial dilutions of phosphate standard solutions with Kinase Dilution Buffer in a volume of 25μl. Also, use 25μl Kinase Dilution Buffer as a blank. The range of phosphate amount should be 0~4 nmol.

Step 3. Thaw the Active VCP on ice. Prepare serial dilutions of VCP using Kinase Dilution Buffer.

Step 4. In a pre-cooled microfuge tube, add the following reaction components in total volume of 25μl:

Component 1. 10μl of diluted Active VCP (Catalog # V116-310G)

Component 2. 5μl of diluted ATP solution (Catalog #A50-09)

Component 3. 10μl Kinase Dilution Buffer IX (Catalog #K29-09)

Step 5. Set up the blank control as outlined in step 4, excluding the addition of the Active ATPase. Replace the Active ATPase with an equal volume of Kinase Dilution Buffer (Catalog # K29-09).

Step 6. Start the reaction by incubating the mixture in a water bath at 37°C for 20 minutes.

Step 7. Add 100μl BIOMOL GREEN Reagent to each reaction including control tubes.

Step 8. Add 100μl BIOMOL GREEN Reagent to each phosphate standard solution including the blank (step 2).

Step 9. Incubate at room temperature for 30 minutes to allow development of the green color.

Step 10. Measure the absorbance of the reaction solution in a spectrophotometer at 650 nm.

Step 11. Plot the free phosphate standard curve. Determine absorbance (y) for each sample (where y = absorbance of sample – background absorbance) and calculate the corresponding nmol phosphate released (x) during the assay using the equation y = A*x + B or x =[y - B] / A (the A and B values are determined from the slope of the line from the standard curve).

Step 12. Calculate the ATPase specific activity (SA):

ATPase Specific Activity (SA) (nmol/min/mg)

SA = Corresponding phosphate released *1000/ [(Reaction time in min)*(Enzyme amount in μg)]

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