Datasheet for ABIN657704

anti-C9 antibody (Center)

1 Validation

3 Images

Go to Product page

Overview

Quantity:	400 µL
Target:	C9
Binding Specificity:	AA 191-220, Center
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This C9 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (Paraffin-embedded Sections) (IHC (p))

Product Details

Immunogen:	This C9 antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 191-220 AA from the Central region of human C9.
Clone:	RB33918
Isotype:	Ig Fraction
Specificity:	This C9 antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 190-220 amino acids from the Central region of human C9.
Purification:	This antibody is purified through a protein A column, followed by peptide affinity purification.

Target Details

Target:	C9

Target Details

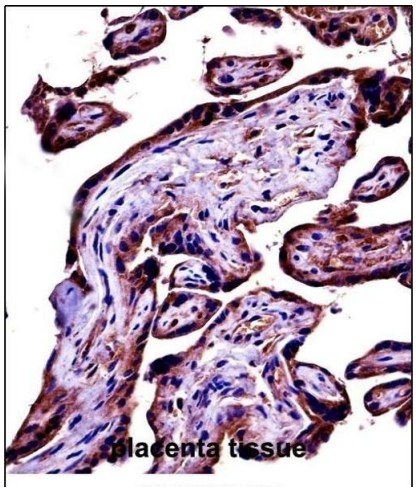
Alternative Name:	C9 (C9 Products)
Background:	This gene encodes the final component of the complement system. It participates in the formation of the Membrane Attack Complex (MAC). The MAC assembles on bacterial membranes to form a pore, permitting disruption of bacterial membrane organization. Mutations in this gene cause component C9 deficiency. [provided by RefSeq]. Synonyms: Complement component C9,C9,
Molecular Weight:	63173 DA
Gene ID:	735
NCBI Accession:	NP_001728
UniProt:	P02748
Pathways:	Complement System

Application Details

Application Notes:	WB = 1:1000, IHC (p) = 1:10-50
Restrictions:	For Research Use only

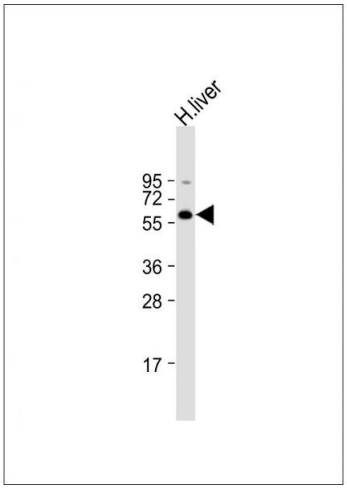
Handling

Format:	Liquid
Concentration:	0.36 mg/mL
Buffer:	PBS with 0.09 % (W/V) sodium azide
Preservative:	Sodium azide
Precaution of Use:	This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C/-20 °C
Storage Comment:	C9 Antibody (Center) can be refrigerated at 2-8 °C for up to 6 months. For long term storage, place the at -20 °C.
Expiry Date:	6 months



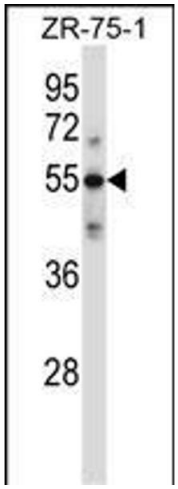
Immunohistochemistry (Paraffin-embedded Sections)

Image 1. C9 Antibody (Center) ((ABIN657704 and ABIN2846695))immunohistochemistry analysis in formalin fixed and paraffin embedded human placenta tissue followed by peroxidase conjugation of the secondary antibody and DAB staining.This data demonstrates the use of C9 Antibody (Center) for immunohistochemistry. Clinical relevance has not been evaluated.



Western Blotting

Image 2. Anti-C9 Antibody (Center) at 1:1000 dilution + human liver lysate Lysates/proteins at 20 µg per lane. Secondary Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/10000 dilution. Predicted band size : 63 kDa Blocking/Dilution buffer: 5 % NFDM/TBST.



Western Blotting

Image 3. C9 Antibody (Center) (ABIN657704 and ABIN2846695) western blot analysis in ZR-75-1 cell line lysates (35 µg/lane).This demonstrates the C9 antibody detected the C9 protein (arrow).

Validation report #102069 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by Protein research group, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense Denmark

Report Number: 102069

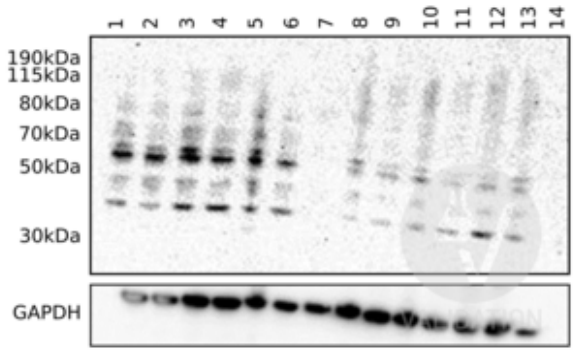
Date: Jan 17 2018

Target:	C9
Method validated:	Western Blotting (WB)
Positive Control:	Human endothelial cells with nanoparticle treatment
Negative Control:	Human endothelial cells and astrocytes without nanoparticle treatment
Notes:	Passed. ABIN657704 specifically recognizes C9 in human endothelial cells.
Primary Antibody:	ABIN657704
Secondary Antibody:	anti-rabbit IgG HRP-linked antibody (Cell Signaling, 7074S)
Protocol:	• Grow human endothelial cells and astrocytes in EndoGRO-MV Complete Media kit (Millipore, SCME004, lot 2885245) supplemented with serum (Millipore, lot 1000958) at 37°C and 5% CO_2 in 8ml on a petri dish to 80-90 % confluency. • Add PVP coated silver nanoparticles (50nm Silver Nanospheres, Nanocomposix USA lot no JRC0234) to the endothelial cells for 24h and 48h. • Lyse 10^6 cells in 100µl per well cold lysis buffer (6M Urea, 2M Thiourea, 10mM DTT). • Determine total protein content of the lysates using Qubit Protein Assay Kit (ThermoFisher Scientific, Q33211). • Denature 20µg of total protein for 5min at 95°C in 5µl Pierce LDS Sample Buffer, Non-Reducing (Thermo Scientific, b31010, lot 151109002) and separate proteins in an XCell SureLock Mini-Cell (Life Technologies, EI0001, lot 007469934) in a Bolt 4-12% Bis-Tris Plus gel (Thermo scientific, NW04125BOX, lot 17120471) for 60min at 100V. • Transfer proteins onto PVDF membrane (Sigma-Aldrich, IPFL00005) with a Western blotting system for 20min at 18V. • Block the membrane with TBST and 5% non-fat milk powder (Sigma-Aldrich, 70166-500G, lot SZBF3420V) for 1h at RT. • Incubation with primary rabbit anti-C9 antibody (antibodies-online, ABIN657704, lot SA110711AL) diluted 1:1000 TBST and 5% non-fat milk powder for ON at 4°C. • Wash membrane 3x for 15min with TBST. • Incubation with secondary anti-rabbit IgG HRP-linked antibody (Cell Signaling, 7074S) diluted 1:10000 in TBST and 5% non-fat milk powder for 2h at RT. • Wash membrane 3x for 15min with TBS. • Reveal protein bands using Luminata Forte Western HRP Substrate (Millipore, WBLUF0100) on a UVP multispectral imaging system (AH diagnostics, Denmark).

- Incubate the membrane in Restore Plus Western blot Stripping Buffer (Thermo Scientific, 46430, lot RL241559) for 20min at RT.
- Wash membrane 3x for 15min with TBST.
- Incubation with rabbit anti-GAPDH antibody (Cell Signaling Technology, 2118) diluted 1:5000 in TBST and 5% non-fat milk powder for 2h at RT.
- Wash membrane 3x for 15min with TBS.
- Reveal protein bands as described above.

Experimental Notes:	Upon addition of the nanoparticles, the endothelial cells' proteome revealed high expression of C9 at the 24h time point and reduced expression at the 48h time point. We validated the proteomics results by western blotting and we confirm that the C9 antibody ABIN657704 reveals a protein of the expected molecular weight (60kDa) in lysates of endothelial cells. The protein band is only visible in the nanoparticle treated cells but not the negative controls.

Image for Validation report #102069



Validation image no. 1 for anti-Complement Component C9 (C9) (AA 191-220), (Center) antibody (ABIN657704)

Immunoblot using ABIN657704 on human endothelial cell and human astrocytes lysates. C9 protein band intensity is increased in the human endothelial cell after 24h (lane 1-6) compared to 48h (lane 8-13) when treated with nanoparticles. Negative controls are endothelial cells (lane 7) and astrocytes (lane 14) without nanoparticle treatment.