

Human Vimentin Profiling ELISA Kit ab173190

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Overview

Product name Human Vimentin Profiling ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Overall	3			7.5%

Inter-assay

Sample	n	Mean	SD	CV%
Overall	3			12%

Sample type

Adherent cells, Suspension cells, Tissue Homogenate, Tissue Lysate

Assay type

Quantitative

Sensitivity

8 µg/ml

Recovery

> 80 %

Sample specific recovery

Sample type	Average %	Range
Cell culture media	80	71% - 87%
Fetal Bovine Serum	89	81% - 93%
Bovine Serum Albumin	89	83% - 84%

Assay duration

Multiple steps standard assay

Species reactivity

Reacts with: Human

Product overview

Abcam's Vimentin Human Profiling *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate qualitative measurement of total vimentin protein in Human samples.

The assay employs an antibody specific to Vimentin coated onto well plate strips. Samples are pipetted into the wells and Vimentin present in the sample is bound to the wells by the

immobilized antibody. The wells are washed and an anti-Vimentin detector antibody is added. After washing away unbound detector antibody, HRP-conjugated label specific for the detector antibody is pipetted into the wells. The wells are again washed, a TMB development solution is added to the wells and blue color develops in proportion to the amount of bound Vimentin.

Notes Vimentin is a type III intermediate filament (IF) protein that forms the cytoskeletal framework in the cytoplasm of eukaryotic cells. Other structural proteins such as actin and tubulin are highly conserved in different cell types, whereas IF proteins are expressed in a highly tissue specific manner. Due to vimentin being the major cytoskeletal protein in mesenchymal cells, it is used as a marker of mesenchymally-derived cells or cells undergoing epithelial-to-mesenchymal transition (EMT) during normal development and metastatic progression.

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Platform Microplate

Properties

Storage instructions Please refer to protocols.

Components	1 x 96 tests
10X Blocking Buffer	1 x 6ml
10X HRP Label	1 x 1ml
10X Vimentin Detector Antibody	1 x 1ml
10X Wash Buffer	1 x 40ml
Extraction Buffer	1 x 15ml
HeLa Whole Cell RIPA Extract	1 x 300µl
HRP Development Solution	1 x 12ml
Vimentin Microplate	1 unit

Function Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally.

Involved with LARP6 in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2.

Tissue specificity Highly expressed in fibroblasts, some expression in T- and B-lymphocytes, and little or no expression in Burkitt's lymphoma cell lines. Expressed in many hormone-independent mammary carcinoma cell lines.

Involvement in disease Cataract 30

Sequence similarities Belongs to the intermediate filament family.

Domain The central alpha-helical coiled-coil rod region mediates elementary homodimerization. The [IL]-x-C-x-x-[DE] motif is a proposed target motif for cysteine S-nitrosylation mediated by the

iNOS-S100A8/A9 transnitrosylase complex.

Post-translational modifications

Filament disassembly during mitosis is promoted by phosphorylation at Ser-55 as well as by nestin (By similarity). One of the most prominent phosphoproteins in various cells of mesenchymal origin. Phosphorylation is enhanced during cell division, at which time vimentin filaments are significantly reorganized. Phosphorylation by PKN1 inhibits the formation of filaments.

Phosphorylated at Ser-56 by CDK5 during neutrophil secretion in the cytoplasm. Phosphorylated by STK33.

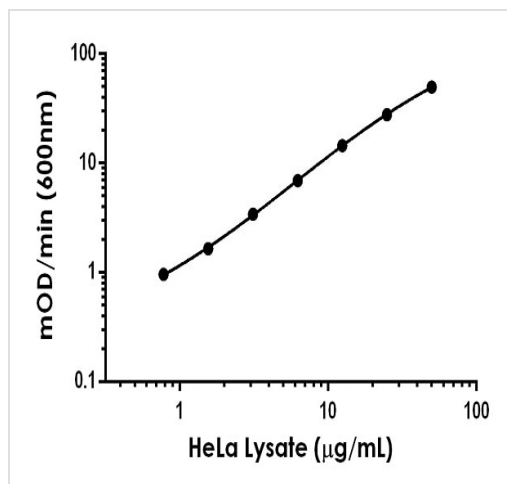
O-glycosylated during cytokinesis at sites identical or close to phosphorylation sites, this interferes with the phosphorylation status.

S-nitrosylation is induced by interferon-gamma and oxidatively-modified low-density lipoprotein (LDL(ox)) possibly implicating the iNOS-S100A8/9 transnitrosylase complex.

Cellular localization

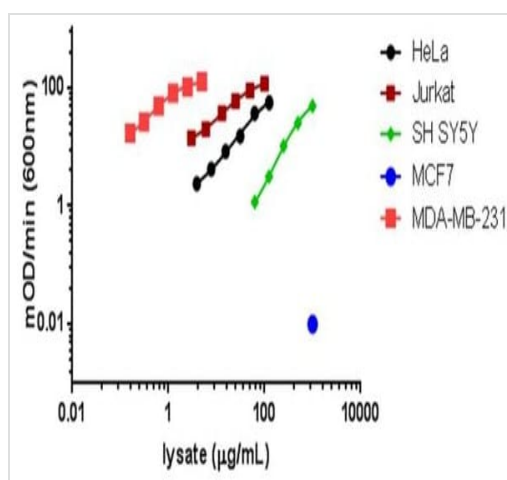
Cytoplasm.

Images



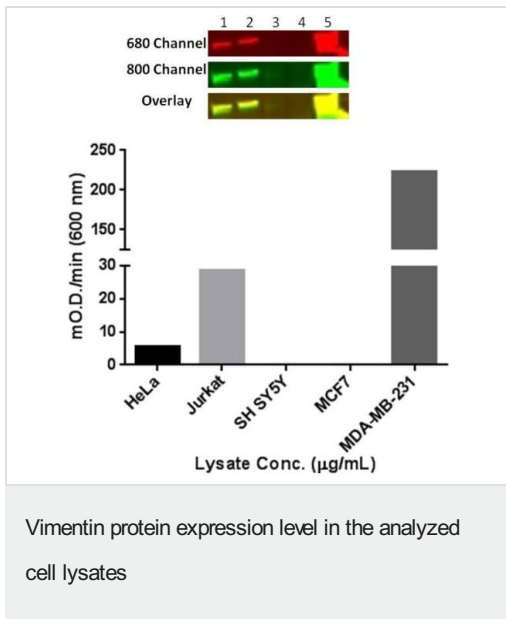
Typical standard curve generated when using the Vimentin Human Profiling ELISA kit.

Typical Standard Curve



HeLa cell lysate, Jurkat cell lysate, SH SY5Y cell lysate, MCF7 cell lysate (not shown) and MDA-MB-231 cell lysate were tested using this kit. HeLa cell lysate shown as standard control sample, varying concentration other cell Lysates were analyzed within working range of the assay.

Experiment showing total Vimentin levels in various cell lines



Western blot of cell lysates (top panel): 1) HeLa, 2) Jurkat, 3) Sy5y, 4) MCF7, 5) MDA-MB-231 (10 µg/lane) using primary antibodies: rabbit anti-vimentin (1/1000 dilution) and mouse anti-vimentin (**ab8978**, 1/1000 dilution); Secondary antibodies used were goat anti-rabbit 680-RD (Red, 1/10,000) and goat anti-mouse 800 (Green, 1/10,000). Blot was scanned using a LIICOR® Odyssey® imager and overlay shows target specificity for total vimentin (yellow). Bar graph (bottom panel) represents relative mOD/min (600 nm) observed for 10 µg/mL cell lysate, except for MDA-MB-231 cell lysate which reports mOD/min (600 nm) for 1 µg/mL.

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