ab173190 – Vimentin Human Profiling ELISA Kit

Instructions for Use

For the measurement of total Vimentin protein in Human samples.

This product is for research use only and is not intended for diagnostic use.

Handling of the stock standard was updated on June 18, 2018.

Version 3a Last Updated 13 January 2023

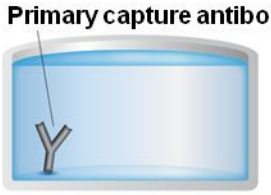
Table of Contents

1. BACKGROUND

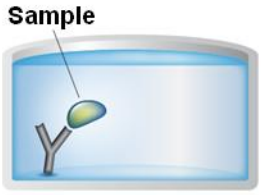
Abcam's Vimentin Human Profiling in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate qualitative measurement of total vimentin protein in Human samples.

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.

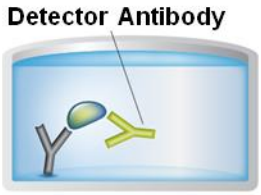
2. ASSAY SUMMARY



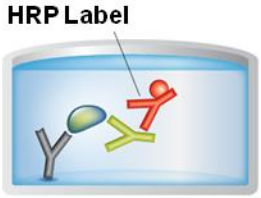
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



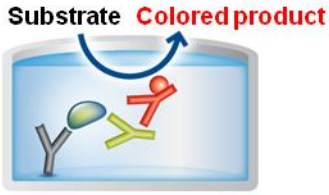
Add standard or sample to each well used. Incubate at room temperature.



Aspirate and wash each well. Add prepared Detector Antibody to each well. Incubate at room temperature.



Aspirate and wash each well. Add prepared HRP label. Incubate at room temperature.



Aspirate and wash each well. Add HRP Development Solution to each well. Immediately begin recording the color development. Alternatively add a Stop solution at a user-defined time.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4ºC immediately upon receipt.

Store HeLa Whole Cell RIPA Extract at -80ºC immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Extraction Buffer	15 mL	4ºC
10X Blocking Buffer	6 mL	4ºC
10X HRP Label	1 mL	4ºC
HRP Development Solution	12 mL	4ºC
10X Wash Buffer	40 mL	4ºC
10X Vimentin Detector Antibody	1 mL	4ºC
Vimentin Microplate (12 x 8 well strips)	96 Wells	4ºC
HeLa Whole Cell RIPA Extract, 3mg/mL	300 µg	-80ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 600 or 450 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi and single channel pipettes.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.3).
- Tubes for standard dilution.
- Stop solution (optional) – 1N Hydrochloric acid (HCl).
- Plate shaker for all incubation steps (optional).
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors) and/or phosphatase inhibitors.

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- Samples generating values higher than the highest positive control sample should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiply freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps (optional).
- When generating positive control samples, it is advisable to change pipette tips after each step.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

9.1 **1X Supplemented Extraction Buffer**

Supplement an appropriate volume of Extraction Buffer with protease inhibitors prior to use. Prepare approximately 500 µL – 2.0 mL of 1X Supplemented Extraction Buffer per sample to be extracted.

9.2 **1X Wash Buffer**

Prepare 1X Wash Buffer by diluting the 10X Wash Buffer 10-fold with nanopure water immediately prior to use. Mix gently and thoroughly. Prepare 15 mL 1X Wash Buffer for each 8 well strip used.

9.3 **1X Vimentin Detector Antibody**

Prepare 1X Vimentin Detector Antibody by diluting the 10X Vimentin Detector Antibody 10-fold with 1X Supplemented Incubation Buffer immediately prior to use. Prepare 500 µL 1X Vimentin Detector Antibody for each 8 well strip used

9.4 **1X HRP Label**

Prepare 1X HRP Label by diluting the 10X HRP Label 10-fold with 1X Supplemented Incubation Buffer immediately prior to use. Prepare 500 µL 1X HRP Label for each 8 well strip used.

9.5 **1X Supplemented Incubation Buffer**

Prepare 1X Supplemented Incubation Buffer by adding 4 mL 10X Blocking Buffer to 36 mL 1X Wash Buffer. Add protease inhibitors according to manufacturer's instructions. Mix gently and thoroughly**.**

- After opening, the unused Incubation Buffer should be stored at -20°C.

10. POSITIVE CONTROL PREPARATION

Prepare a set of serially diluted positive controls using the frozen HeLa lysate provided with the kit immediately prior to use. Always prepare a fresh set of positive controls for every use.

10.1 Thaw the HeLa Whole Cell RIPA Extract on ice. This is the 3000 µg/mL **Stock Solution**. This stock solution should be aliquoted and stored at -80□C after first use to avoid additional freeze-thaw cycles

10.2 Label tubes numbers 1-8 and add 491.7 µL of 1X Supplemented Incubation Buffer to tube #1 and 150 µL 1X Supplemented Incubation Buffer into tubes #2-8.

10.3 To generate **Standard #1** add 8.3 µL of **Stock Solution** to tube #1. Mix gently but thoroughly.

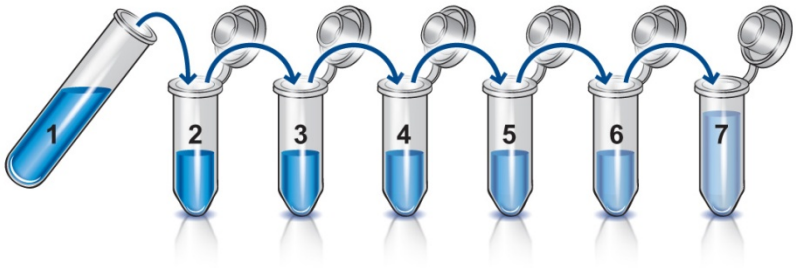
10.4 To generate **Standard #2** add 150 µL Standard #1 to tube #2. Mix gently but thoroughly.

10.5 To generate **Standard #3** transfer 150 µL from Standard #2 to tube #3. Mix gently but thoroughly.

10.6 Using the table below as a guide, repeat for tubes #4 through #7.

10.7 Tube #8 containing only 1X Supplemented Incubation Buffer should be used as a zero control.

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (µg/mL)	Final Conc. (µg/mL)
1	Stock	8.3	491.7	3000	50
2	Standard #1	150	150	50	25
3	Standard #2	150	150	25	12.5
4	Standard #3	150	150	12.5	6.25
5	Standard #4	150	150	6.25	3.12
6	Standard #5	150	150	3.12	1.56
7	Standard #6	150	150	1.56	0.78



11. SAMPLE PREPARATION

TYPICAL SAMPLE DYNAMIC RANGE -

Sample Type	Range (µg/mL)
HeLa	8 – 125
Jurkat	6 – 100
Sy5Y	63 – 1,000
MDA-MB-231	0.16 - 5.0

11.1 Preparation of extracts from cell pellets

11.1.1 Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4ºC.

11.1.2 Rinse cells twice with PBS.

11.1.3 Solubilize cell pellet at $2x10^7$/mL in 1X Supplemented Extraction Buffer.

11.1.4 Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.2 Preparation of extracts from adherent cells by direct lysis (alternative protocol)

11.2.1 Remove growth media and rinse adherent cells 2 times in PBS.

11.2.2 Solubilize the cells by addition of 1X Supplemented Extraction Buffer directly to the plate (use 750 µL - 1.5 mL 1X Supplemented Extraction Buffer per confluent 15 cm diameter plate).

11.2.3 Scrape the cells into a test tube and incubate the lysate on ice for 15 minutes. Centrifuge at

18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

11.3 **Preparation of extracts from tissue homogenates**

11.3.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

11.3.2 Homogenize 100 to 200 mg of wet tissue in 500 µL - 1 mL of the supplied 1X Supplemented Extraction Buffer. For lower amounts of tissue adjust volumes accordingly.

11.3.3 Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and seal.

13.3 Add 50 µL of each serially diluted HeLa lysate standard per well. It is recommended to include a dilution series of a control sample as a reference. It is important to include a 1X Supplemented Incubation Buffer as a zero standard.

13.4 Cover/seal the plate and incubate for 2 hours at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.

13.5 Aspirate each well and wash, repeat this once more for a total of **two** washes. Wash by aspirating or decanting from wells then dispensing 300 µL 1X Wash buffer into each well as described above. Complete removal of liquid at each step is essential to good performance. After the last wash, remove the remaining buffer by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

13.6 Immediately before use prepare sufficient (500 µL/strip used) 1X Vimentin Detector Antibody (step 9.3) in 1X Supplemented Incubation Buffer. Add 50 µL 1X Detector antibody to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.

13.7 Repeat the aspirate/wash procedure above.

13.8 Immediately before use prepare sufficient (500 µL/strip used) 1X HRP Label in 1X Supplemented Incubation Buffer (step 9.4). Add 50 µL 1X HRP label to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.

13.9 Repeat the aspirate/wash procedure above, however, performing a total of **three** washes.

13.10 Add 50 µL HRP Development Solution to each empty well and immediately record the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode:	**Kinetic:**
Wavelength:	600 nm
Time:	up to 15 min.
Interval:	10 sec. - 1 min.
Shaking:	Shake before and between readings

Alternative– In place of a kinetic reading, at a ***user defined***, time record the endpoint OD data at (i) 600 nm or (ii) stop the reaction by adding 50 µL Stop Solution to each well and record the OD at 450 nm.

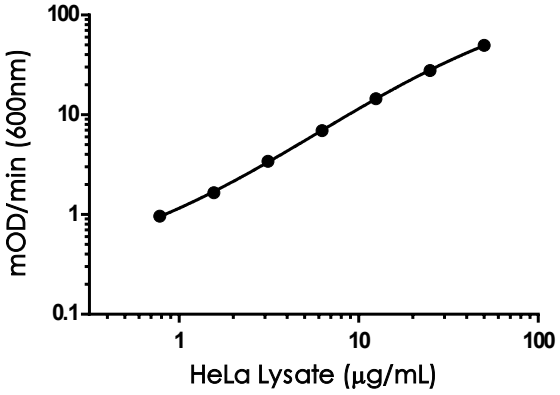
13.11 Analyze the data as described below.

14. CALCULATIONS

Subtract average zero standard reading from all readings. Average the duplicate readings of the positive control dilutions and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Read relative protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Standard Curve Measurements			
Conc (µg/mL)	mO.D./min		
	1	2	Mean
0	2.399	2.115	2.257
0.781	3.166	3.161	3.163
1.562	4.072	3.665	3.868
3.125	5.497	5.763	5.630
6.25	9.055	9.255	9.155
12.5	16.625	16.843	16.734
25	29.885	30.005	29.945
50	51.380	52.293	51.837

Figure 1. Typical standard curve generated using the Vimentin Human Profiling in ELISA kit. Kinetic readings at 600 nm for 10 minutes.

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

Calculated Minimum Detectable Dose	Zero does + 2 Standard Deviations
HeLa Lysate	<0.39 µg/mL

RECOVERY –

(Sample spiking in representative sample matrices)

Sample Type	Average % Recovery	Range %
50% Culture Media	80	71 – 87
10% BSA	89	83 – 94
10% FBS	89	81 – 93

LINEARITY OF DILUTION –

Dilution Factor	Jurkat Cell Lysate (µg/mL)	Interpolated HeLa Cell Lysate Standard (µg/mL)	% Expected Value
Undiluted	50	277	100
2	25	128	92
4	12.5	70	101
8	6.125	37	107

Dilution Factor	MDA-MB-231 Cell Lysate (µg/mL)	Interpolated HeLa Cell Lysate Standard (µg/mL)	% Expected Value
Undiluted	2.5	409	100
2	1.25	223	109
4	0.63	100	98
8	0.31	49	96

Dilution Factor	SH SY5Y Cell Lysate (µg/mL)	Interpolated HeLa Cell Lysate Standard (µg/mL)	% Expected Value
Undiluted	1000	100	100
2	500	46	92
4	250	20	80

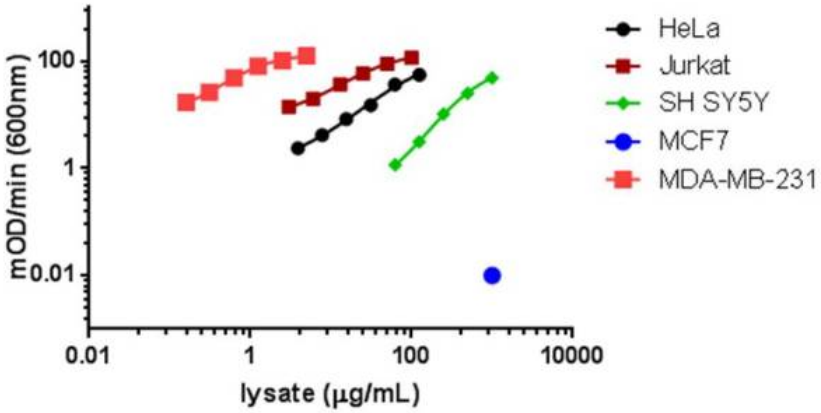


Figure 2A. Experiment showing total vimentin levels in HeLa cell lysate, Jurkat cell lysate, SH SY5Y cell lysate, MCF7 cell lysate (not shown) and MDA-MB-231 cell lysate. HeLa cell lysate shown as standard control sample, varying concentration other cell Lysates were analyzed within working range of the assay.

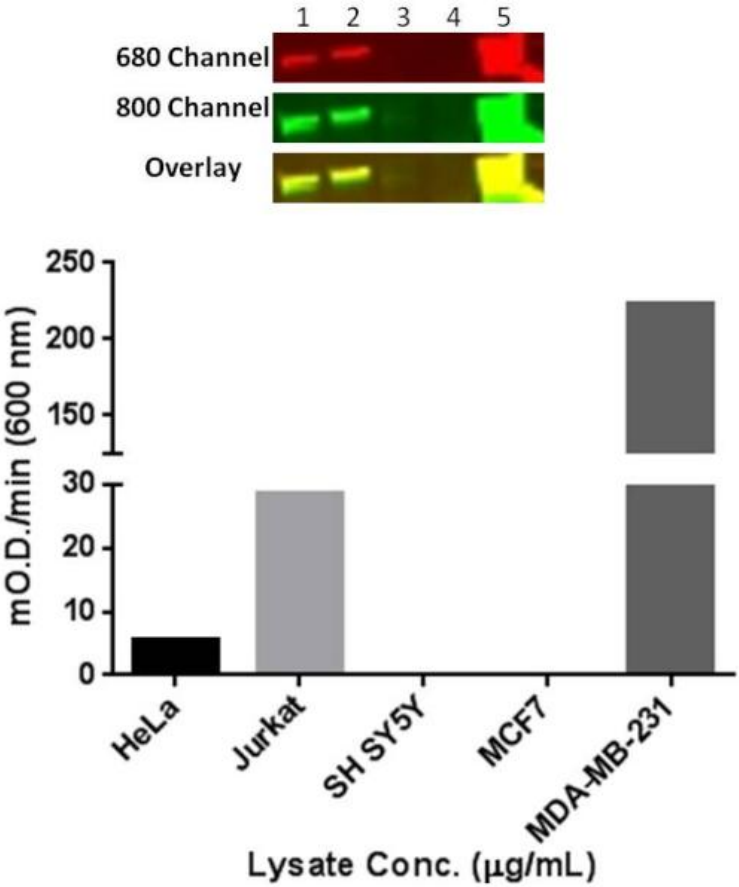


Figure 2B. The total vimentin protein expression level in the analyzed cell lysates. Western blot of cell lysates (top panel): 1) HeLa, 2) Jurkat, 3) Sy5y, 4) MCF7, 5) MDA-MB-231(10 µg/lane) using primary antibodies: rabbit anti-vimentin (ab92547, 1/1,000 dilution) and mouse anti-vimentin (ab8978, 1/1000 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 LI-COR® 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.

PRECISION –

	Intra-Assay	Inter-Assay
n=	3	3
%CV	7.5	12.0

17. SPECIES REACTIVITY

This kit detects total Vimentin in **Human samples only**.

18. TROUBLESHOOTING

Problem	Cause	Solution
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
Large CV	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Incubation times with HRP solution too brief	Ensure sufficient incubation time till blue color develops prior addition of Stop solution
	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
Low sensitivity	Contaminated wash buffer	Prepare fresh wash buffer
	Improper storage of the ELISA kit	Store all assay components 4°C. Keep substrate solution protected from light

19. **NOTES**

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 Version 3a |2023-01-12