VIM (Human) ELISA Kit

Catalog Number KA3127

96 assays

Version: 24

Intended for research use only

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Introduction

Intended Use

For quantitative detection of Vimentin in human serum, plasma, urine, cell culture supernatant or tissue samples.

Background

Vimentin is a type III intermediate filament (IF) protein that is expressed in mesenchymal cells. IF proteins are found in all metazoan cells as well as bacteria. All IF proteins are expressed in a highly developmentally-regulated fashion; vimentin is the major cytoskeletal component of mesenchymal cells. Because of this, vimentin is often used as a marker of mesenchymally-derived cells or cells undergoing an epithelial-to-mesenchymal transition (EMT) during both normal development and metastatic progression. It plays a significant role in supporting and anchoring the position of the organelles in the cytosol, and it has been used as a sarcoma tumor marker to identify mesenchyme.

Principle of the Assay

This kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. The purified anti-Vimentin antibody was pre-coated onto 96-well plates. And the HRP conjugated anti-Vimentin antibody was used as detection antibodies. The standards, test samples and HRP conjugated detection antibody were added to the wells subsequently, mixed and incubated, then, unbound conjugates were washed away with wash buffer. TMB substrates (A & B) were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Vimentin amount of sample captured in plate. Read the O.D. absorbance at 450 nm in a microplate reader, and then the concentration of Vimentin can be calculated.

General Information

Materials Supplied

List of component

Component	Amount
One 96-well plate pre-coated with anti-human Vimentin antibody	96 wells
Standard (900 ng/mL)	0.5 mL
Standard diluent buffer	1.5 mL
Wash buffer (30x), Dilution: 1:30	20 mL
Sample diluent buffer	6 mL
HRP conjugated anti-human Vimentin antibody (RTU)	6 mL
Stop solution	6 mL
TMB substrate A	6 mL
TMB substrate B	6 mL
Plate sealer	2 slices
Hermetic bag	1 bag

Storage Instruction

Store at 2-8°C for 6 months.

Materials Required but Not Supplied

- ✓ 37°C incubator
- ✓ Microplate reader (wavelength: 450 nm)
- ✓ Precise pipette and disposable pipette tips
- ✓ Automated plate washer
- ✓ ELISA shaker
- ✓ 1.5 mL of Eppendorf tubes
- ✓ Absorbent filter papers
- ✓ Plastic or glass container with volume of above 1 L

Precautions for Use

- ✓ For research use only. Not for diagnostic and clinical use.
- ✓ Before the experiment, centrifuge each kit component for several minutes to bring down all reagents to the bottom of tubes.
- ✓ It is recommend to measure each standard and sample in duplicate.
- ✓ Do NOT let the plate completely dry at any time! Since the dry condition can inactivate the biological material on the plate.
- ✓ Do not reuse pipette tips and tubes to avoid cross contamination.
- ✓ Do not use the expired components and the components from different batches.
- ✓ Store the TMB substrate B in dark.
- ✓ Prolong the incubation time if the hypochromasia obtained. Heat the water in the water bath during diluting if the crystalloid appeared in Wash buffer.
- ✓ Do not remove microplate from the storage bag until needed, and the unused strips should be stored at 2-8°C in their pouch or the provided Hermetic bag.

Assay Protocol

Reagent Preparation

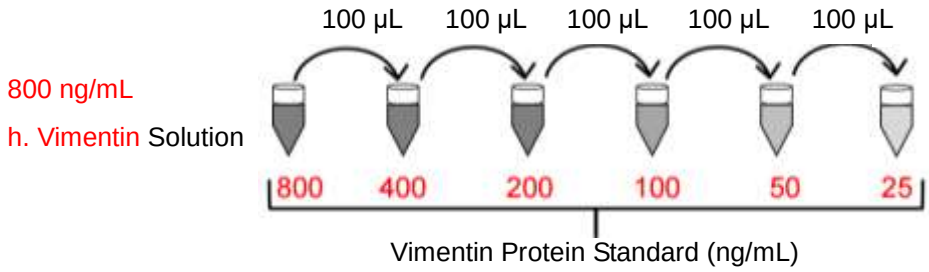
- ✓ Wash buffer

  Dilute concentrated Wash buffer 30-fold (1:30) with distilled water (i.e. add 20 mL of concentrated wash buffer into 580 mL of distilled water).

- ✓ Standard

  Dilution of the human Vimentin standard: standard solution should be prepared no more than 2 hours prior to the experiment. (*Note: Do not dilute the standard directly in the plate*)

  - 800 ng/mL of standard solution: Add 200 µL of the 900 ng/mL standard into 25 µL Standard diluent buffer and mix thoroughly.
  - 400 ng/mL → 25 ng/mL of standard solutions: Label 5 Eppendorf tubes with 400 ng/mL, 200 ng/mL, 100 ng/mL, 50 ng/mL, 25 ng/mL, respectively. Aliquot 100 µL of the Standard diluent buffer into each tube. Add 100 µL of the above 800 ng/mL standard solution into 1st tube and mix thoroughly. Transfer 100 µL from 1st tube to 2nd tube and mix thoroughly. Transfer 100 µL from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. Avoid repeated freeze-thaw cycles.

Sample Preparation

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- ✓ Serum: Coagulate at room temperature for 10-20 min, then, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. If precipitation appeared, centrifuge again.
- ✓ Plasma: Collect plasma using EDTA or citrate plasma as an anticoagulant, and mix for 10-20 min, centrifuge at the speed of 2000-3000 r.p.m. for 20 min of collection. If precipitation appeared, centrifuge again.
- ✓ Urine: Collect urine using a sterile container, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. If precipitation appeared, centrifuge again. For collection of hydrothorax and cerebrospinal fluid, take reference to this operation.
- ✓ Cell culture supernatant: For secretory components: use a sterile container to collect. Centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant. For intracellular components: Dilute cell

suspension with PBS (pH7.2-7.4) to make the cell concentration reached 1 million/mL. Damage cells and release of intracellular components through repeated freeze-thaw cycles. Centrifuge at the speed of 2000-3000 r.p.m. For 20 min to collect supernatant. If precipitation appeared, centrifuge again.

- ✓ Tissue samples: Cut samples and weight, add certain volume of PBS (pH7.4), rapidly frozen with liquid nitrogen. After melting, store samples at 2-8°C. Add certain volume of PBS (pH7.4), homogenize thoroughly, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant.

  Note 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particle.

  Note 2. NaN_3 can not be used as test sample preservative, since it is the inhibitor for HRP.

  Note3. After collecting samples, analyze immediately or aliquot and store frozen at -20°C. Avoid repeated freeze-thaw cycles.

Assay Procedure

1. Equilibrate kit components for 15-30 min at room temperature.
2. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. Add 50 µL of diluted standards (800 ng/mL, 400 ng/mL, 200 ng/mL, 100 ng/mL, 50 ng/mL, 25 ng/mL) into the standard wells. Add 50 µL of Standard diluent buffer into the control (zero) well. Do not add sample and HRP conjugated antibody into the control (zero) well.
3. For test sample wells, add 40 µL of Sample diluent buffer first, then, add 10 µL of sample. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Cover the plate with Plate sealer and incubate at 37°C for 30 min.
5. Remove the sealer, and wash plate using one of the following methods:
   - ✓ Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers. Fill each well completely with Wash Buffer (1x) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers. Repeat this procedure four more times for a total of FIVE washes.
   - ✓ Automated Washing: Aspirate all wells, then wash plates FIVE times using Wash Buffer (1x). After the final wash, invert plate, and clap the plate on absorbent filter papers until no moisture remained. It is recommended that the washer be set for a soaking time of 10 seconds or shaking.
6. Add 50 µL of HRP conjugated anti-human Vimentin antibody into each well (except control well).
7. Cover the plate with Plate sealer and incubate at 37°C for 30 min.
8. Remove the sealer, and wash the plate. (See Step 5)
9. Add 50 µL of TMB substrate A into each well, and then, add 50 µL of TMB substrate B, vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds), and incubate in dark at 37°C for 15 min. The shades of blue can be seen in the wells.
10. Add 50 µL of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
11. Read the O.D. absorbance at 450 nm in a microplate reader within 15 min after adding the stop solution.

Data Analysis

Calculation of Results

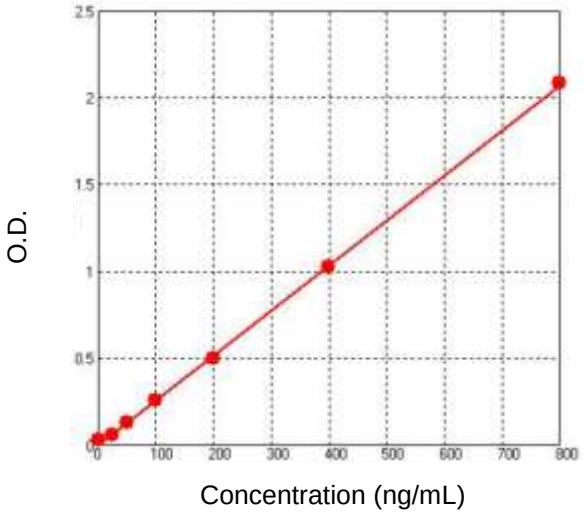
For calculation, (the relative O.D.$_{450}$) = (the O.D.$_{450}$ of each well) – (the O.D.$_{450}$ of Zero well). The standard curve can be plotted as the relative O.D.$_{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Vimentin concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

✓ Typical Data and Standard Curve

Results of a typical standard run of VIM (Human) ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	ng/mL	0	25	50	100	200	400	800
Y	OD450	0.032	0.062	0.128	0.256	0.520	1.024	2.084



Performance Characteristics

✓ Range: 20 ng/mL - 800 ng/mL

Resources

References

1. Leader M, Collins M, Patel J, Henry K (January 1987). "Vimentin: an evaluation of its role as a tumour marker". Histopathology 11 (1): 63 – 72.
2. Eriksson JE, Dechat T, Grin B, Helfand B, Mendez M, Pallari HM, Goldman RD (2009). "Introducing intermediate filaments: from discovery to disease". J Clin Invest 119 (7): 1763 – 71.
3. Cabeen MT, Jacobs-Wagner C (2010). "The bacterial cytoskeleton". Annu Rev Genet 44: 365 – 92.

Plate Layout

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