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Human KDR/VEGFR2 ELISA Kit

Catalog No: CKH193

Size: 1 x 96 tests

Introduction:

Kinase Insert Domain Protein Receptor (KDR), better known as VEGF Receptor 2 (VEGFR2), is one of the two receptors of VEGF and is a Type III Receptor Tyrosine Kinase (RTK). It functions as the main mediator of VEGF-induced endothelial proliferation, survival, migration, tubular morphogenesis, and sprouting (i.e., cord formation). The signaling and trafficking of KDR/VEGFR2 are regulated by multiple factors, including Rab GTPase, P2Y Purine Nucleotide Receptor, Integrin alpha V beta 3, and T-cell Protein Tyrosine Phosphatase. Mutations of the KDR/VEGFR2 gene are implicated in infantile capillary hemangiomas.

The Human KDR/VEGFR2 ELISA Kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of Human KDR/VEGFR2 in serum, plasma, cell culture supernatants, and urine. This assay employs an antibody specific for KDR/VEGFR2 coated on a 96-well plate. Standards and samples are pipetted into the wells and KDR/VEGFR2 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and Biotinylated Anti-Human KDR/VEGFR2 antibody is added. After washing away unbound Biotinylated antibody, HRP-Streptavidin is pipetted into the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of KDR/VEGFR2 bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Performance and Characteristics:

Sensitivity

The minimum detectable dose of KDR/VEGFR2 is typically less than 70 pg/ml.

Reproducibility

Intra-Assay: CV<10%
Inter-Assay: CV<12%

Recovery

Recovery was determined by spiking various levels of Human KDR/VEGFR2 into Human serum, plasma, and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	96.46	85-105
Plasma	94.78	84-103
Cell culture media	96.23	86-104

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Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	95	94	96
	Range (%)	83-103	84-104	85-103
1:4	Average % of Expected	97	95	101
	Range (%)	85-104	84-104	86-105

Reagents and materials supplied in the kit:

Items	Quantity
A. Microplate coated with Anti-Human KDR/VEGFR2 (12 strips x 8 wells)	96 wells
B. Wash Buffer Concentrate (20x)	25 mL
C. Recombinant Human KDR/VEGFR2 Standards	2 vials
D. Assay Diluent A: Standard/Sample-Serum/Plasma *	30 mL
E. Assay Diluent B (5x): Standard/Sample-Cell Culture Medium/Urine	15 mL
F. Detection Antibody: Biotinylated Anti-Human KDR/VEGFR2	2 vials
G. Streptavidin-HRP Concentrate (600x)	200 µl
H. TMB One-Step Substrate Reagent (TMB in buffered solution)	12 mL
I. Stop Solution (0.2 M Sulfuric Acid)	8 mL

** Contains 0.09% Sodium Azide as preservative. Precaution: Sodium Azide is a poisonous and hazardous substance which should be handled by trained staff only.*

Storage of Kit Reagents:

Stable for up to 6 months from date of shipment at 2-4°C. Store reconstituted standard (recombinant protein) at -80°C. Opened Microplate Wells and reagents are stable for 1 month at 2-4°C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge.

Materials/reagents required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- 100 ml and 1 liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard or sample dilutions

Preparation of Kit Reagents:

Bring all reagents and samples to room temperature (18-25°C) before use.

Sample Dilution

If your samples need to be diluted, use Assay Diluent A (Item D) for dilution of serum/plasma samples, and Assay Diluent B (Item E) for dilution of culture supernatants/urine.

Suggested dilution for normal serum/plasma: 10-100 fold*.

*Please note that levels of target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

Assay Diluent B

Dilute 5-fold with deionized or distilled water.

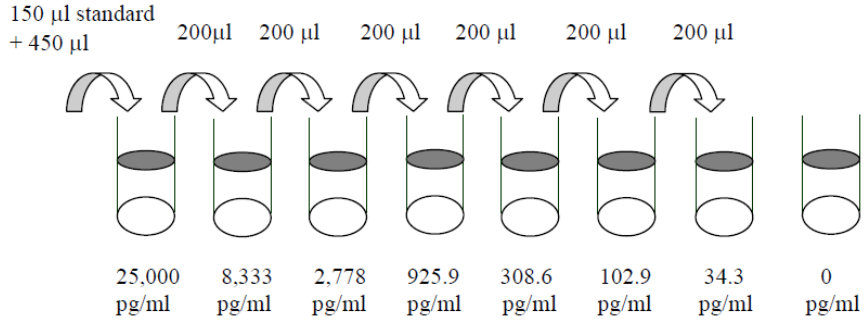
Wash Buffer Concentrate

- If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved.
- Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

KDR/VEGFR2 Standard

- Briefly spin the vial of Item C (Recombinant Human KDR/VEGFR2 Standard).
- Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium/urine) into Item C to prepare a 100 ng/ml standard.
- Dissolve the powder thoroughly by a gentle mix.
- Add 150 µl standard from the vial of Item C into a tube with 450 µl Assay Diluent A or 1x Assay Diluent B to prepare a 25,000 pg/ml stock standard solution.
- Pipet 400 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below in Figure 1).
- Mix each tube thoroughly before the next transfer.
- Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).

Figure 1



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Detection Antibody

- Briefly spin Detection Antibody vial (Item F) before use.
- Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
- Pipet up and down to mix gently (the concentrate can be stored at 2-4°C for 5 days).
- The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of the **ELISA Method**.

Streptavidin-HRP Concentrate

- Briefly spin Streptavidin-HRP Concentrate vial (Item G) and pipette up and down to mix gently before use.
- Streptavidin-HRP concentrate should be diluted 600-fold with 1x Assay Diluent B.

For example: Briefly spin the vial (Item G) and pipet up and down to mix gently. Add 20 µl of Streptavidin-HRP concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a 600-fold diluted Streptavidin-HRP solution (do not store the diluted solution for next day use). Mix thoroughly.

ELISA Method:

Be sure to read **'Preparation of Kit Reagents'** before carrying out the assay

1. Bring all reagents and samples to room temperature (18-25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see **Preparation of Kit Reagents: KDR/VEGFR2 Standard**) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared biotinylated antibody (see **Preparation of Kit Reagents: Detection Antibody**) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3
6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents: Streptavidin-HRP Concentrate**) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Assay Procedure Summary:

1. Prepare all reagents, samples and standards as instructed.

⇩

2. Add 100 µl standard or sample to each well.
   Incubate 2.5 hours at room temperature or overnight at 2-4°C.

⇩

3. Add 100 µl prepared biotin antibody to each well.
   Incubate 1 hour at room temperature.

⇩

4. Add 100 µl prepared Streptavidin solution.
   Incubate 45 minutes at room temperature.

⇩

5. Add 100 µl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.

⇩

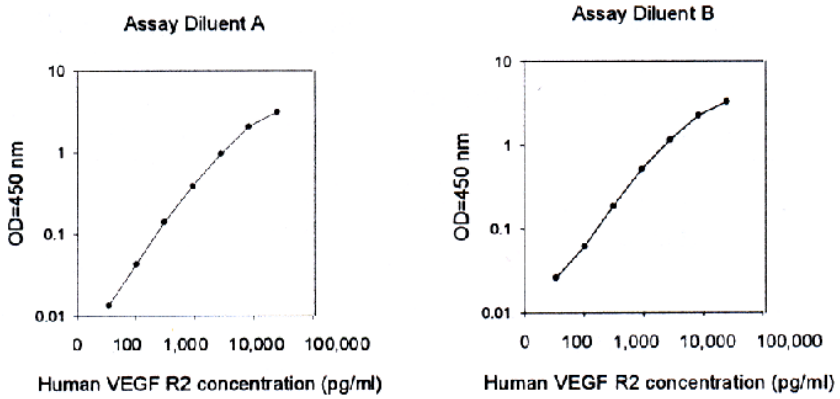
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data:

Standard curve is for demonstration **ONLY**. A standard curve **MUST** be run with each assay.



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Troubleshooting Guide:

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times	1. Ensure sufficient incubation time; ELISA Method Step 2 may change to overnight.
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation.
3. Large CV	1. Inaccurate pipetting	1. Check pipettes.
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer.
5. Low sensitivity	1. Improper storage of the ELISA Kit	1. Store your standard at <-20°C after reconstitution, others at 2-4°C. Keep substrate solution protected from light.
	2. Stop solution	2. Stop solution should be added to each well before measurement.

Specificity:

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested *(e.g.*, Angiopoietin 1, Angiostatin , BMP7, CD14, CD30, CD40, CD40 Ligand, CTLA4, CXCL16, Dkk-4 , DR6, Endostatin, E-Selectin, Follistatin, HB-EGF, HVEM, ICAM-2, IGF2, IL-10 Ra, IL-10 Rb, IL-18, IL-9 , IL-2 Ra, IL-2 Rb, IL-5 Ra, LAP, L-Selectin, M-CSF R, MMP-1,-2, -3, -7, -8, -9, -10 and -12, PDGF-AB, SDF-1b, Tie-1, Tie-2, TIMP-3.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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