# Human Vascular endothelial growth factor receptor 2 (FLK1/KDR) ELISA Kit

**Catalog No.:** abx050238

**Size:** 96T

**Range:** 30 pg/ml - 800 pg/ml

**Sensitivity:** < 10 pg/ml

**Storage:** Store at 2-8°C for 6 months.

**Application:** For quantitative detection of FLK1 in Human Serum, Plasma, Cell Culture Supernatants, Tissue Homogenates and urine.

**Introduction:** Kinase insert domain receptor (KDR, a type III receptor tyrosine kinase) also known as vascular endothelial growth factor receptor 2 (VEGFR-2) is a VEGF receptor. KDR is the human gene encoding it. Kinase insert domain receptor has been shown to interact with SHC2, Annexin A5 and SHC1.

**Principle of the Assay**

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. Anti-FLK1 antibody is pre-coated onto a 96-well plate. An HRP conjugated antibody is used as detection antibody. The standards, test samples and HRP conjugate reagent are added to the wells and incubated. Unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the FLK1 amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of FLK1 can be calculated.

**Kit components**

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Human FLK1 Standard: 0.5 ml
3. Standard diluent buffer: 1.5 ml
4. Wash buffer (30×): 20 ml. Dilution: 1/30
5. Sample diluent buffer: 6 ml
6. HRP Conjugate Reagent: 6 ml
7. Stop solution: 6 ml
8. TMB substrate A: 6 ml
9. TMB substrate B: 6 ml
10. Plate sealer: 2
11. Hermetic bag: 1

**Material Required But Not Provided**

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. High-precision pipette and sterile pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

## Protocol

### A. Preparation of sample and reagents

#### 1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Allow the serum to clot in a serum separator tube (about 30 min) at room temperature. Centrifuge at approximately 1000 x g for 10 min. Analyze the serum immediately or aliquot and store frozen at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA as the anticoagulant. Centrifuge for 15 min at 1000 × g within 30 min of collection. Analyze immediately or aliquot and store frozen at - 20 °C or -80°C.
- **Cell culture supernate, tissue lysate or body fluids:** Remove particulates by centrifugation, analyze immediately or aliquot and store frozen at -20°C or -80°C.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Urine:** Aseptically collect the first urine of the day (mid-stream) voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:**

» Samples must be diluted so that the expected concentration falls within the kit's range.

» Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» $NaN_3$ cannot be used as test sample preservative, since it inhibits HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

#### 2. Wash buffer

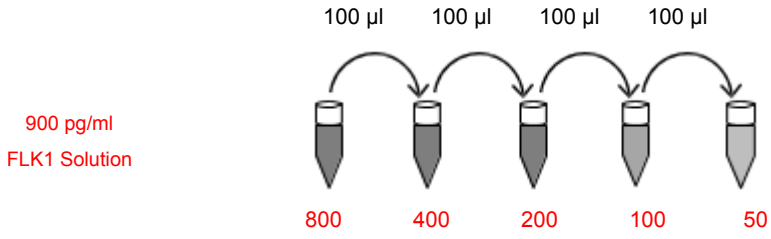
Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

#### 3. Standard

The standards should be prepared no more than 2 hours before the experiment. (Note; do not dilute standard directly in the plate).

1. 800 pg/ml standard. Add 200 µl of the 900 pg/ml standard into 25 µl of the standard diluent buffer and mix thoroughly.

2. 400 pg/ml → 50 pg/ml standard solutions. Label 4 Eppendorf tubes with 400 pg/ml, 200 pg/ml, 100 pg/ml and 50 pg/ml. Aliquot 100 µl of the Standard diluent buffer into each tube. Add 100 µl of the above 800 pg/ml standard solution into the first tube and mix thoroughly. Transfer 100 µl from the first tube to the second tube and mix thoroughly, and so on.




### B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each

test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively and record their positions.
2. Aliquot 50 µl of the diluted standards into the standard wells.
3. Aliquot 50 µl of Standard diluent buffer into the control (zero) well. Do not add sample and HRP Conjugate Reagent into the control (zero) well.
4. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly to mix the contents.
5. Seal the plate with a cover and incubate at 37°C for 30 min.
6. Remove the cover and wash the plate 5 times with one of the following methods.

**Manual Washing:** Discard the solution without touching the side walls. Fill each well completely (approximately 400 µl) with Wash buffer and incubate on an ELISA shaker for 2 min. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. Repeat this procedure for a total of five times.

**Automated Washing:** Discard the solution and wash the plate fie times overfilling the wells with Wash buffer. After the final wash invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. It is recommended that the washer be set for a soaking time of 1-2 min.

7. Aliquot 50 µl of HRP Conjugate Reagent into each well (except control well).
8. Seal the plate with a cover and incubate at 37°C for 30 min.
9. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 6.
10. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.
11. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation, (relative O.D.450) = (O.D.450 of each well) – (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). Log-log curve fitting is recommended for data analysis. The Human FLK1 concentration of the samples can be interpolated from the standard curve.

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

**C. Precautions**

1. Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. It is recommend to measure each standard and sample in duplicate or triplicate.

5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
6. Ensure that the plate is properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. To avoid cross contamination do not reuse pipette tips and tubes.
9. Do not use expired components or components from a different kit.
10. Store the TMB substrate B in the dark and to avoid edge effect of plate incubation for temperature differences it is recommended to equilibrate the TMB substrates for 30 min at room temperature prior to use. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.