Human/Monkey VEGFR3/FLT4 ELISA Kit

Instructions for Use | Cat. No.: AEHY0276

1. Content

Component	CAT	Volume
CP (Coated Plate)	EHY0276CP	96 well
S (Standard)	EHY0276S1~S7,S0	8 vial
DD (Detect Antibody Diluent)	EDD02	6 ml/bottle
DA-H (Detect Antibody-HRP 100×)	EHY0276DA-H	50 µl/1 vial
AB (Assay Buffer 1×)	EAB01	12 ml/bottle
TS (TMB Substrate)	ETS01	12 ml/bottle
SS (Stop Solution)	ESS01	12 ml/bottle
WB (Wash Buffer 10×)	EWB01	50 ml/bottle
SF (Sealer Film)	ESF01	6 pieces

NOTE: After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

2. Reagent Preparation

Washing Buffer (1×) Preparation

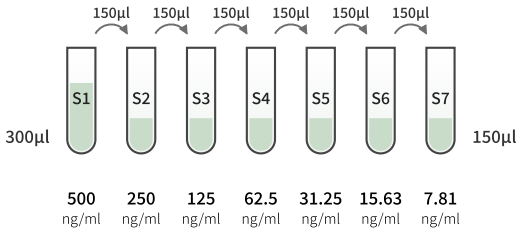
Pour entire contents (50 ml) of the Washing Buffer Concentrate (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25°C.

1×DA Preparation

Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated Detect Antibody solution with DD (Detect Antibody Diluent) in a clean plastic tube as needed according to the Standards and Samples.

Standard Curve Preparation

S1 to S7 and S0 is ready to use for serum and plasma.

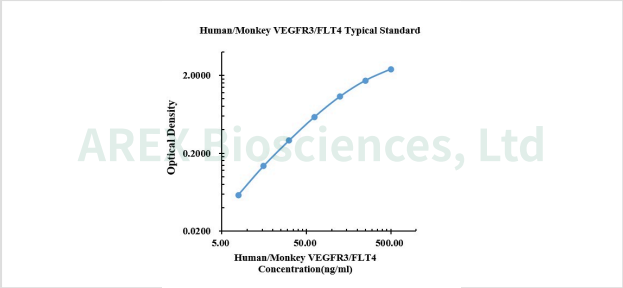


3. Assay Procedure

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.
3. Add 50 µl of **AB** (Assay Buffer) to each well.
4. Add 10 µl of Standard or sample per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.
5. Add 50 µl of **DA**-H (Detect Antibody-HRP) to each well.
6. Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25°C) for 30 min on a microplate shaker set at 500 rpm.
7. Aspirate each well and wash, repeating the process four times. Wash by filling each well with **WB** (Washing Buffer 300 µl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining **WB** (Washing Buffer) by aspirating or decanting. Invert the plate and blot it against clean paper towels.
8. Add 100 µl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.
9. Add 100 µl of **SS** (Stop Solution) to each well.
10. Determine the optical density within 30 minutes, using microplate reader set to 450 nm corrected with 570 nm or 630 nm.

4. Typical Data



(ng/ml)	O.D.	Average	Corrected
0.00	0.0035 / 0.0032	0.0034	-
7.81	0.0605 / 0.0614	0.0610	0.0578
15.63	0.1381 / 0.1410	0.1396	0.1364
31.25	0.2995 / 0.2867	0.2931	0.2899
62.50	0.5641 / 0.6069	0.5855	0.5823
125.00	1.0540 / 1.0820	1.0680	1.0648
250.00	1.7850 / 1.6480	1.7165	1.7133
500.00	2.4030 / 2.4070	2.4050	2.4018

5. Sensitivity

The minimum detectable dose (MDD) of human/monkey VEGFR3/FLT4 is typically less than 0.10 ng/ml (10 µl of sample volume).
The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

6. Precision

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty-two times on one plate to assess intra-assay precision.
Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested six times on one plate to assess intra-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample Number	S1	S2	S3	S1	S2	S3
	22	22	22	6	6	6
Average(ng/ml)	7.3	38.1	128.0	8.3	40.4	128.0
Standard Deviation	0.3	1.8	6.3	0.4	1.8	6.1
Coefficient of Variation(%)	4.6	4.7	4.9	4.9	4.5	4.8

7. Recovery

The spike recovery was evaluated by spiking 3 levels of human/monkey VEGFR3/FLT4 into healthy human/monkey serum sample.
The un-spiked serum was used as blank in this experiment. The recovery ranged from 91% to 117% with an overall mean recovery of 101%.

8. Linearity

To assess the linearity of the assay, five samples were spiked with high concentration of VEGFR3/FLT4 in human/monkey serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.
The linearity ranged from 95% to 115% with an overall mean recovery of 105%.

9. Sample Values

Serum/Plasma – Thirty samples from apparently healthy volunteers and two samples from healthy monkeys were evaluated for the presence of human/monkey VEGFR3/FLT4 in this assay. No medical histories were available for the donors.

Sample Matrix	Sample Evaluated	Range (ng/ml)	Detectable %	Mean of Detectable (ng/ml)
Human Serum	30	26.62-155.63	100	101.03
Monkey Serum	2	100.08-251.00	100	175.54

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.