Human/Monkey VEGFR3/FLT4 ELISA Instructions

Cat:EHY0276

Content

	CAT	Volume
❶ CP (Coated Plate)	EHY0276CP	96 well
❷ S (Standard)	EHY0276S1~S7,S0	8 vial
❸ DD (Detect Antibody Diluent)	EDD02	6 ml/bottle
❹ DA-H (Detect Antibody-HRP 100×)	EHY0276DA-H	50 μl/1 vial
❺ AB (Assay Buffer 1×)	EAB01	12 ml/bottle
❻ TS (TMB Substrate)	ETS01	12 ml/bottle
❼ SS (Stop Solution)	ESS01	12 ml/bottle
❽ WB (Wash Buffer 10×)	EWB01	50 ml/bottle
❾ 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.

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℃.

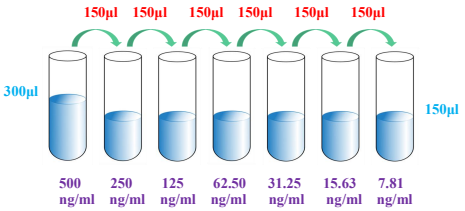
1×DA Preparation:

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.



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

❶ Prepare all reagents and working standards as directed in the previous sections.

❷ Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.

❸ Add 50 μl of **AB** (Assay Buffer) to each well.

❹ Add 10 μl of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.

❺ Add 50 μl of **DA-H** (Detect Antibody-HRP) to each well.

❻ Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 30 min on a microplate **shaker** set at 500 rpm.

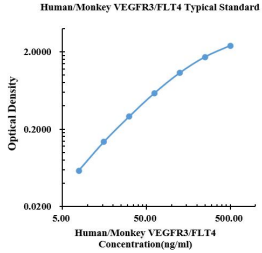
❼ Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 μl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.

❽ Add 100 μl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.

❾ Add 100 μl of **SS** (Stop Solution) to each well.

❿ Determine the optical density within 30 minutes, using microplate **reader** set to 450 nm corrected with 570 nm or 630 nm.

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

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.

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)

	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

RECOVERY

The spike recovery was evaluated by spiking 3 levels of human/monkey VEGFR3/FLT4 into health 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%.

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%.

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.