# Human MERTK ELISA Instructions

## Cat:EHY0189

## Content

| | CAT | Volume |
|---|---|---|
| ❶ CP (Coated Plate) | EHY0189CP | 96 well |
| ❷ S (Standard) | EHY0189S1 | 2 vial |
| ❸ DA-H (Detect Antibody-HRP) | EHY0189DA-H | 50 μl/vial |
| ❹ SD (Sample Diluent) | ESD01 | 15ml/bottle |
| ❺ DD (Detect Antibody Diluent) | EDD02 | 6 ml/bottle |
| ❻ AB (Assay Buffer 1×) | EAB01 | 12 ml/bottle |
| ❼ TS (TMB Substrate) | ETS01 | 12 ml/bottle |
| ❽ SS (Stop Solution) | ESS01 | 12 ml/bottle |
| ❾ WB (Wash Buffer 10×) | EWB01 | 50 ml/bottle |
| ❿ 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

■ **Standard Curve Preparation:**

Reconstitute human MERTK Standard by addition of distilled water as S1. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 200ng/ml).

Allow the standard to reconstitute for 10-30 minutes. Mix well prior to making dilutions.

The human MERTK Standard EHY0189S1 as the high standard (200ng/ml). Pipette 200 μl of SD into each tube. Use the high standard to produce a 1:2 dilution series. Mix each tube thoroughly before the next transfer. SD serves as the zero standard (0 ng/ml).
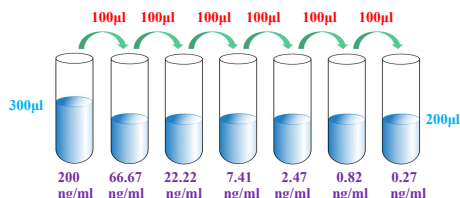


■ **Washing Buffer (1×) Preparation**

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.

## 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 50 μl or 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

**Human MERTK  Typical Standard**
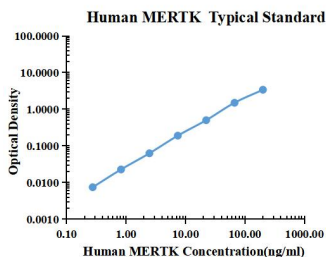


| ng/ml | O.D. | | Average | Corrected |
|-------|------|------|---------|-----------|
| 0.00 | 0.0114 | 0.0112 | 0.0113 | |
| 0.27 | 0.0191 | 0.0184 | 0.0188 | 0.0075 |
| 0.82 | 0.0343 | 0.0338 | 0.0341 | 0.0228 |
| 2.47 | 0.0751 | 0.0732 | 0.0742 | 0.0629 |
| 7.41 | 0.1987 | 0.2044 | 0.2016 | 0.1903 |
| 22.22 | 0.4883 | 0.5378 | 0.5131 | 0.5018 |
| 66.67 | 1.4530 | 1.5690 | 1.5110 | 1.4997 |
| 200.00 | 3.4070 | 3.4100 | 3.4085 | 3.3972 |

## SENSITIVITY

The minimum detectable dose (MDD) of human MERTK is typically less than 0.03 ng/ml (50 µl of sample volume) or 0.41 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 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） | 3.7 | 21.9 | 76.7 | 3.0 | 17.4 | 58.8 |
| Standard Deviation | 0.2 | 0.9 | 4.1 | 0.2 | 1.1 | 4.5 |
| Coefficient of Variation （%） | 5.5 | 4.0 | 5.3 | 5.6 | 6.1 | 7.7 |

## RECOVERY

The spike recovery was evaluated by spiking 3 levels of human MERTK into health human serum sample. The un-spiked serum was used as blank in this experiment.

The recovery ranged from 70% to 105% with an overall mean recovery of 94%.

## LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of MERTK in human serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 74% to 97% with an overall mean recovery of 82%.

## SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy volunteers were evaluated for the presence of human MERTK in this assay. No medical histories were available for the donors.

| Sample Matrix | Sample Evaluated | Range (ng/ml) | Detectable % | Mean of Detectable (ng/ml) |
|---|---|---|---|---|
| Serum | 30 | 37.02-124.26 | 100 | 56.40 |

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