



# QuickDetect™ T cell receptor (TCR) (Human) ELISA Kit

03/19

( Catalog # E4705-100; 96 assays; Storage at 4°C )

## I. Introduction:

BioVision's Human T cell receptor (TCR) ELISA kit is based on Sandwich ELISA method. The Micro Elisa strip plate provided in this kit has been pre-coated with an antibody specific to TCR. Standards or samples are added to the appropriate Micro Elisa strip plate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP) conjugated antibody specific for TCR is added to each Micro Elisa strip plate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. The wells that contain TCR and HRP conjugated TCR antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of TCR. You can calculate the concentration of TCR in the samples by comparing the OD of the samples to the standard curve.

## II. Applications:

Detection range: 5 pg/mL - 2000pg/mL

Assay Precision: Intra-Assay: CV<10%, Inter-Assay: CV<12%, CV (%) = SD/mean X 100

Sensitivity: 10 pg/mL

## III. Sample Type:

- Plasma
- Cell and tissue culture supernatants
- Serum
- Other biological fluids

## IV. Kit Contents:

Components	E4705-100	Part Number
Micro ELISA strip-plate	1	E4705-100-1
Standard (4000 pg/ml)	0.5 ml	E4705-100-2
Standard diluent	6 ml	E4705-100-3
HRP-Conjugate reagent	10 ml	E4705-100-4
Sample diluent	6 ml	E4705-100-5
Chromogen Solution A	6 ml	E4705-100-6
Chromogen Solution B	6 ml	E4705-100-7
Stop Solution	6 ml	E4705-100-8
Wash solution (20X)	25 ml	E4705-100-9
Plate sealers	2	E4787-100-10

## V. User Supplied Reagents and Equipment:

- Distilled or deionized water
- Microplate reader capable of measuring absorbance at 450 nm

## VI. Storage Conditions and Reagent Preparation:

The entire kit may be stored at 4°C in dark for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.

**Note:** Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

**Wash Buffer:** Dilute the concentrated washing buffer (20X) with distilled water.

**Standard Preparation:** Ten wells are set for standards in a Micro Elisa strip plate. In Well 1 and Well 2, 50 µl Standard solution and 50 µl Standard Dilution buffer are added and mixed well. In Well 3 and Well 4, 50 µl solution from Well 1 and Well 2 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50 µl solution from Well 3 and Well 4 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. In Well 7 and Well 8, 50 µl solution from Well 5 and Well 6 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50 µl solution from Well 7 and Well 8 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 9 and Well 10. After dilution, the total volume in all the wells is 50 µl and the concentrations are 2000 pg/mL, 1000 pg/mL, 500 pg/mL, 250 pg/mL and 125 pg/mL, respectively.

**Sample Preparation:** Note: Sample extraction and ELISA assay should be performed as soon as possible after sample collection. If ELISA assay cannot be performed immediately, samples can be stored at -20°C. Avoid multiple freeze-thaw cycles. Samples with Na<sub>3</sub> should be avoided for this assay.

**Serum samples:** After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the



sample should be centrifuged again.

**Plasma sample:** Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifuged for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifuged again.

**Urine samples:** Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuged again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

**Cell samples:** If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuged again.

**Tissue samples:** Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80°C for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4°C. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

**Note:** End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

#### VII. Assay Protocol:

**Note:** Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VI.
2. Add 100  $\mu$ l HRP-Conjugate reagent to each well except the blank control well. Incubate 60 min at 37°C.:
3. In sample wells, add 40  $\mu$ l Sample dilution buffer and 10  $\mu$ l samples are added (dilution factor is 5). Leave a well empty as blank control. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
4. Remove plate sealer, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
5. Add 50  $\mu$ l Chromogen Solution A and 50  $\mu$ l Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes in dark.:
6. Add 50  $\mu$ l stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
7. Read absorbance O.D. at 450nm within 15 minutes after adding stop solution. The OD value of the blank control well is set as zero.

**Calculation:** Known concentrations of Human TCR Standard and its corresponding reading OD is plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human TCR in sample is determined by plotting the sample's O.D. on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

#### VIII. Related Products:

TCR alpha Antibody (5648)

TCR beta Antibody (5641)

**FOR RESEARCH USE ONLY! Not to be used on humans.**