



QuickDetect™ dsDNA (Human) ELISA Kit

rev 02/21

(Catalog # E4484-100, 100 assays, Store at 4°C)

I. Introduction:

BioVision's dsDNA ELISA kit is a sandwich ELISA assay for the qualitative measurement of dsDNA in human serum, plasma, cell culture supernatants and biological fluids in 90 minutes. The Micro ELISA strip-plate provided in this kit has been pre-coated with an antibody specific to dsDNA which reacts with the standards or samples added to the wells. HRP-conjugated antibody specific for dsDNA was added to which react with TMB substrate to generate color. The concentration of dsDNA in the samples can be quantified by comparing the OD of the samples with the standard.

II. Application:

This ELISA kit is used for *in vitro* qualitative determination of dsDNA.

Cross Reactivity: No significant cross-reactivity or interference between this analytes and its analogues was observed.

Assay range: 0.1 IU/mL - 60 IU/mL

Sensitivity: 1.0 IU/mL

III. Specificity:

Human

IV. Sample Type:

Serum, plasma, urine, cell culture samples, biological fluid

V. Kit Contents:

Components	E4484-100	Part No.
Micro ELISA strip-plate	1	E4484-100-1
Standard (96 IU/mL)	0.5 ml	E4484-100-2
Standard diluent	6 ml	E4484-100-3
HRP- Conjugate reagent	10 ml	E4484-100-4
Sample diluent	6 ml	E4484-100-5
Chromogen Solution A	6 ml	E4484-100-6
Chromogen Solution B	6 ml	E4484-100-7
Stop Solution	6 ml	E4484-100-8
Wash buffer (20X)	25 ml	E4484-100-9
Plate sealers	2	E4484-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator

VII. Storage and Handling:

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.

VIII. Reagent Preparation:

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.

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

2. Standard Preparation:

10 wells are set for standards in a Micro Elisa strip-plate. In Well 1 and Well 2, 50 µl Standard solutions and 50 µl Standard Dilution buffer are added and mixed well. In Well 3 and Well 4, 50µl solution from Well 1 and Well 2 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 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, 50µl 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 48 IU/mL, 24 IU/mL, 12 IU/mL, 6 IU/mL and 3 IU/mL respectively.

3. 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 NaN₃ should be avoided for this assay.

- **Serum:** 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 centrifuge again.

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



- **Plasma:** 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 centrifuge again.
- **Urine:** 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 centrifuge 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 centrifuge 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.
- 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.

IX. 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 anti-dsDNA antibody assay.

1. In the Micro Elisa strip plate, leave two wells as blank control. In sample wells add 40 µl **Sample dilution buffer** and 10µl **sample** (dilution factor is 5). Mix well with gentle shaking.
2. Add 100 µl **HRP-Conjugate reagent** to each well, except blank well.
3. Incubate 60 min at 37°C after sealed with Closure plate membrane.
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 µl **Chromogen Solution A** and 50 µl **Chromogen Solution B** to anti-dsDNA antibody well, mix with gently shaking and incubate at 37°C for 15 minutes in dark..
6. Add 50 µl **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.

X. DETERMINE THE RESULT:

Plot known concentrations of Human dsDNA Standard and its corresponding reading OD on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of Human dsDNA 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.

Precision: Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Human dsDNA were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Human dsDNA were tested on 3 different plates, 8 replicates in each plate.

$CV (\%) = SD/mean \times 100$

Intra-Assay: CV<10%

Inter-Assay: CV<12%

XI. RELATED PRODUCTS:

- Anti-DNA antibody (Mouse) ELISA Kit (E4355-100)
- EZQuant™ dsDNA Quantitation Kit (Fluorometric) (K900-100)
- QuickDetect™ Anti-single stranded DNA (Human) ELISA Kit (E4483-100)

