Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

QuickDetect™ Anti-ssDNA Antibody (Rat) ELISA Kit

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(Catalog # E4485-100, 100 assays, Store at 4°C)

I. Introduction:

Antibodies against single-stranded DNA are mainly directed against its basic compound, which in the native DNA is masked inside the helical structure. Anti-ssDNA antibody (Ab) test is used for the differential diagnosis of new patients suspected of an inflammatory rheumatic disease the clinical sensitivity for systemic lupus erythematosus (SLE). The increase in anti-ssDNA Ab level appeared to be the best predictor of forthcoming increase in anti-dsDNA and SLE flare BioVision's Anti-ssDNA ELISA kit is a sandwich ELISA assay for the qualitative measurement of Anti-ssDNA antibody in rat serum, plasma and cell culture supernatants in 90 minutes.

II. Application:

This ELISA kit is used for *in vitro* qualitative determination of Anti-ssDNA.
Cross Reactivity: No significant cross-reactivity or interference between this analyte and its analogues was observed.

III. Specificity:

Rat

IV. Sample Type:

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

V. Kit Contents:

Components	E4485-100	Part No.
Micro ELISA strip-plate	1	E4485-100-1
Negative control	0.5 ml	E4485-100-2
Positive control	0.5 ml	E4485-100-3
HRP- Conjugate reagent	6 ml	E4485-100-4
Sample diluent	6 ml	E4485-100-5
Chromogen Solution A	6 ml	E4485-100-6
Chromogen Solution B	6 ml	E4485-100-7
Stop Solution	6 ml	E4485-100-8
Wash buffer (30X)	20 ml	E4485-100-9
Plate sealers	2	E4485-100-10

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 (30X) with distilled water.
2. **Sample Preparation:**

Note: Sample extraction and ELISA assay should be performed as soon as possible after sample collection. If ELISA assay can not be performed immediately, samples can be stored at -20°C. Avoid multiple freeze-thaw cycles. Samples with NaN_3 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.
- **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℃. 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-ssDNA antibody assay.

1. In the Microelisa stripplate, leave two wells as negative control, two wells as positive control and one well empty as blank control. Number the sequential number, corresponding sample of the microporous hole 2 per board should set negative control and positive control 2 wells, and one well without samples and HRP-Conjugate reagent, the rest of the same step operation.
2. Adding samples: Negative and positive control in a volume of 50 µl are added to the negative and positive control wells respectively. In sample wells, 40 µl Sample dilution buffer and 10 µl sample are added. Mix well with gentle shaking.
3. Incubate 30 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 HRP-Conjugate reagent to anti-ssDNA antibody well except the blank control well. Incubate 30 min at 37°C.
6. Washing as described in Step 4.
7. Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to anti-ssDNA antibody well, mix with gently shaking and incubate at 37°C for 15 minutes in dark.
8. Add 50 µl stop solution to well to terminate the reaction. The color in the well should change from blue to yellow.
9. 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:

Test effectiveness: the average value of positive control ≥1.00; the average value of negative control ≤0.10. The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15. Negative judgement: if the OD value< CUT OFF, the sample is Rat Anti-ssDNAnegative. Positive judgement: if the OD value ≥CUT OFF, the sample is Rat Anti-ssDNA positive.

XI. RELATED PRODUCTS:

- Anti-DNA antibody (Mouse) ELISA Kit (E4355-100)
- EZQuant™ dsDNA Quantitation Kit (Fluorometric) (K900-100)
- QuickDetect™ Anti-double stranded DNA (Rat) ELISA Kit (E4484-100)
- QuickDetect™ Anti-single stranded DNA (human) ELISA Kit (E4483-100)