ab287819 – Human Anti-ssDNA Antibody ELISA Kit

For in vitro qualitative determination of Anti-ssDNA in human Serum, plasma, cell culture samples, and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab287819

Storage and Stability

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.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA strip-plate	1	4°C
Negative control	0.3 ml	4°C
Positive control	0.3 ml	4°C
HRP- Conjugate reagent	10 ml	4°C
Sample diluent	6 ml	4°C
Chromogen Solution A	6 ml	4°C
Chromogen Solution B	6 ml	4°C
Stop Solution	6 ml	4°C
Wash buffer (20X)	25 ml	4°C
Plate sealers	2	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- 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.

Sample Preparation

- Samples with NaN3 should be avoided for this assay.

Δ 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.*

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 centrifuged again.

Plasma: Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubation at room temperature for 10-20 minutes, centrifuge the tubes 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: 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 samples.

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 should be 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 are homogenized after adding PBS (pH 7.4). Samples should be handled 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.

Δ Note: *End users 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.*

Assay Protocol

- 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 Micro ELISA strip-plate, reserve two wells as negative controls, two wells as positive controls, and leave one well empty as a blank control. Number each remaining well with sequential numbers for identifying corresponding samples, using 2 wells per board for each sample. The blank control should have no sample and no HRP-Conjugate reagent added, but should follow the same steps as the rest of the operation.
2. Adding samples: 50 µl of negative and positive controls should be added to the negative and positive control wells respectively. In the sample wells, add 40 µl Sample dilution buffer and 10 µl sample. Mix well with gentle shaking.
3. Add 100 µl HRP-Conjugate reagent to each well, except the blank control well. Seal the plate, and incubate for 60 min at 37°C.
4. Remove the plate sealer, aspirate the contents of each well, 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 the wells, mix with gentle shaking, and incubate at 37°C for 15 minutes in the dark.
6. Add 50 µl stop solution to the wells to terminate the reaction. The color in the well should change from blue to yellow.
7. Read absorbance O.D. at 450 nm within 15 minutes of adding the stop solution. The OD value of the blank control well is set as zero.

Calculation

Test effectiveness: the average value of positive control ≥1.00; the average value of negative control is ≤0.15. The critical value (CUT OFF) is calculated as follows:

critical value = the average value of negative controls + 0.15

Negative Result: if the Sample OD value < CUT OFF, the sample is Human Anti-ssDNA negative.
Positive Result: if the Sample OD value ≥ CUT OFF, the sample is Human Anti-ssDNA positive.

Technical Support

For all technical or commercial enquiries please go to:
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