



# ANA Screen (Antinuclear Antibody) ELISA Kit

rev06/19

( Catalog # E4708-100; 96 assays; Storage at 4°C )

## I. Introduction:

Antinuclear antibodies (ANA) are frequently present in patients with systemic lupus erythematosus (SLE) and, less commonly, in other autoimmune diseases Rheumatoid arthritis, Collagen vascular diseases, chronic liver diseases and systemic sclerosis (scleroderma). ANA bind to several nuclear antigens including DsDNA, SSDNA, RNP, Sm, SSA and SSB. ANA frequency increases with age in apparently healthy people, especially women after the age of 45 years. ANA ELISA is widely used as a screening procedure for different autoimmune diseases. BioVision's ANA Screen ELISA test system is an enzyme-linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to ANA in human serum or plasma. Diluted patient serum is added to the ELISA plate coated with purified nuclear antigens. ANA IgG specific antibody binds to the antigen present in the samples. The enzyme conjugate is added to bind to the antibody-antigen complex. After addition of substrate the plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

## II. Applications:

- Detection of Antinuclear Antibody in human serum samples

## III. Sample Type:

- Plasma
- Serum

## IV. Kit Contents:

Components	E4708-100	Part Number
Microwells coated with nuclear antigens	12 x 8	E4708-100-1
Sample Diluent	22 ml	E4708-100-2
Calibrator	1 ml	E4708-100-3
Positive Control	1 ml	E4708-100-4
Negative Control	1 ml	E4708-100-5
Enzyme conjugate	12 ml	E4708-100-6
TMB Substrate	12 ml	E4708-100-7
Stop Solution	12 ml	E4708-100-8
Wash buffer (20X)	25 ml	E4708-100-9

## 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.

**Wash buffer:** Prepare 1X Wash buffer by adding to 475 ml of distilled or deionized water to 25 ml (20X) buffer. Store at room temperature.

**Sample preparation:** Collect blood specimens and separate the serum.

Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.

## 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.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of sample diluent. Mix well.
3. Add 100 µl of diluted **serum samples**, **calibrator**, and **controls** into the appropriate wells. For blank, add 100 µl **sample diluent** in the well. Tap the plate to remove air bubbles and mix well. Incubate for 20 minutes at room temperature.



4. Remove liquid from all wells. Wash wells three times with 300  $\mu$ l of 1X wash buffer. Blot on paper towel.
5. Dispense 100  $\mu$ l of **enzyme conjugate** to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300  $\mu$ l of 1X wash buffer. Blot on paper towel
7. Dispense 100  $\mu$ l of **TMB substrate** and incubate for 10 minutes at room temperature.
8. Add 100  $\mu$ l of **Stop solution**.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with a reference filter of 600-650 nm.

**Calculation:** Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.

1. **Calculate the cut-off value:** Calibrator OD x Calibrator Factor (CF).
2. Calculate the Antibody Index of each determination by dividing the O.D. value of each sample by cut-off value.

**VIII. Related Products:**

- C-Peptide ELISA Kit (E4701)
- Creatinine (Human) ELISA Kit (E4368)
- C-Reactive Protein (CRP) (Human) ELISA Kit (E4289)
- Alpha-Fetoprotein (AFP) ELISA (K4242)

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