Signosis
Innovative Plate Assay Solutions

ANA ELISA Kit

Catalog Number EA-5013 **(For Research Use Only)**

Introduction

Anti-nuclear antibodies (ANA) are a group of antibodies directed against various nuclear and some cytoplasmic antigens. Although these antibodies were first associated with systemic lupus erythematosus (SLE), the list of implicated diseases has expanded and many rheumatic diseases are characterized by the presence of one or more of these ANAs. For instance, anti-SSA/Ro and anti-SSB/La antibodies are associated with SLE and Sjogren's Syndrome (SS), anti-dsDNA and anti-Sm antibodies with SLE, anti-histone antibodies with SLE and Drug Induced Lupus, anti-RNP antibodies with mixed connective tissue disease (MCTD) and SLE, anti-Scl-70 antibodies with scleroderma (progressive systemic sclerosis (PSSJ), anti-Jo1 with polymyositis and dermatomyositis and anti-centromere antibodies with CREST syndrome. ANA are usually detected by indirect immunofluorescence (IFA) on HEp-2. Because of certain limitations of IFA, ANA ELISA test is more robust offering several advantages including ease of operation and not requiring skills needed to perform and read IFA reactions. ANA ELISA test is able to efficiently screen large numbers of patient samples and reduces human error. As ANA ELISA test collectively detects, in one well, total ANAs against double stranded DNA (dsDNA), Histones, SS-A/Ro, SS-B/La, Sm, Sm/RNP, Scl-70, Jo-1, and centromeric antigens, more specific antibody tests are recommended to perform in patients with positive ANA.

Principle of the assay

ANA ELISA kit measures anti-nuclear antibodies in the serum. It is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes extracted antigens from HEp-2 cells for immobilization on the microtiter wells and anti-human IgG antibody conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two components, resulting in ANA being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of ANA is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

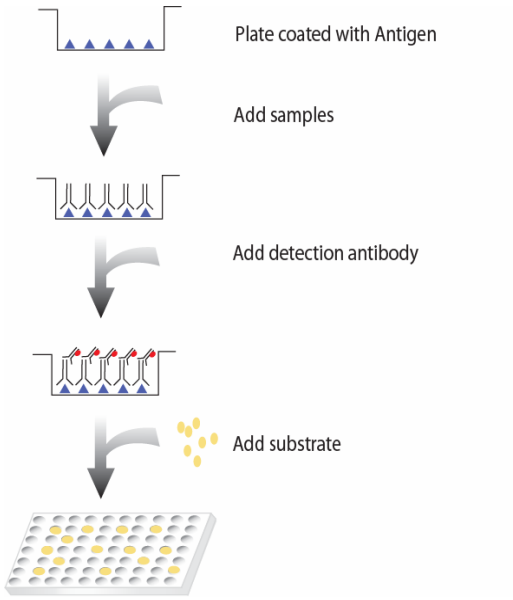

Diagram of ELISA

Materials provided with the kit

- 8x12 96-well plate coated with antigens from Hep-2 cells (4ºC).
- Anti-human IgG antibody conjugated to HRP (4ºC).
- Positive control (-20ºC)
- 1X Diluent buffer (4ºC).
- 5X Assay wash buffer (4ºC).
- Substrate (4ºC).
- Stop Solution (4ºC)

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Shaker

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 1000 times of anti-human IgG antibody conjugated to HRP with 1X Diluent buffer.

Storage and Preparation

Store all reagents at 2-8°C.
All reagents must be brought to room temperature (20-25°C) prior to use.
When stored at 2-8°C, the diluted Assay wash buffer is stable until the kit expiration date.

Storage and Preparation

Detection Range: 40 pg/ul – 2500 pg/ul
Limit of Detection: 10 pg/ul

Precautions

Human blood derivatives and patient specimens should be considered potentially infectious. All human derived components need to be tested for the negative HBsAg, HCV, HIV-1 and 2 and HTLV-I. Follow good laboratory practices in storing, dispensing and disposing of these materials.

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Add 100µl of diluent buffer to the wells to be used. Then add 1µl of sample or positive control directly in the well to make a 1:100 dilution. Incubate for 1 hour at room temperature with gentle shaking. *Note: We recommend having a blank condition. For the blank, add only diluent buffer to the well.
3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process twice for a total of three washes. Completely remove liquid at each wash by firmly tapping the plate against clean paper towels.
4. Add 100µl of diluted anti-human IgG antibody conjugated to HRP to each well and incubate for 30 minutes at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100µl of Substrate to each well and incubate for 5-30 minutes.
7. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
8. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.