



Eight-ANA ELISA Screen Kit

Catalog Number EA-5101

(For Research Use Only)

Introduction

Anti-nuclear antibodies (ANA) are a group of antibodies directed against various nuclear and some cytoplasmic antigens. Serological tests for ANA play an important role towards the diagnosis of various autoimmune connective tissue disorders. 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-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. As ANA ELISA test collectively detects, in one well, total ANAs against double stranded DNA (dsDNA), Sm, U1-RNP (68K), SS-A/Ro, SS-B/La, Scl-70, Jo-1, and centromeric antigens, along with sera positive for IFA HEp-2 ANAs, it is not specific indicators of a connective tissue disease. To monitor more specific antibodies, eight different antigens (dsDNA, Smd1, U1-RNA (68K), SS-A/Ro, SS-B/La, Scl-70, Jo-1, and CENP-B) are coated to different wells in a column or strip for the ELISA screen test of eight different autoimmune antibodies once.

Principle of the assay

In eight-ANA ELISA screen test, eight different antigens (dsDNA, Smd1, U1-RNP (68K), SS-A/Ro, SS-B/La, Scl-70, Jo-1, and CENP-B) are coated into different wells in a column or strip. In each well of the strip, one specific antigen is coated for monitoring a corresponding antibody and therefore total 8 wells of a strip allow measurement of 8 different antibodies. The test sample is allowed to react simultaneously with the two components, resulting in anti-nuclear antibodies 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 anti-nuclear antibodies is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

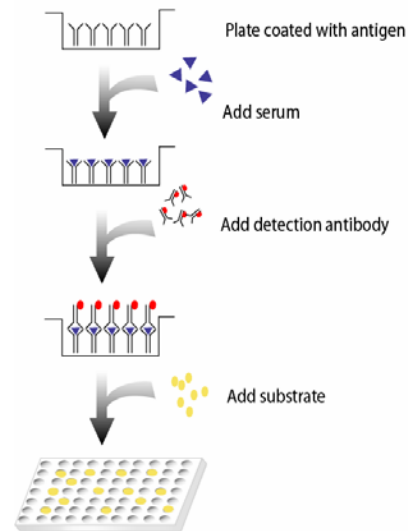


Diagram of ELISA

Materials provided with the kit

- 96-well plate coated with 8 different antigens (4°C).
- Anti-human IgG antibody conjugated to HRP (4°C).
- 40ml 1X Diluent buffer (4°C).
- 40ml 5X Assay wash buffer (4°C).
- 10ml Substrate (4°C).
- 6 ml Stop Solution (4°C)

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 500 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.

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. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
2. Add 100 µl of diluted samples (1:100 diluted or further 2 serial diluted serum) per well and incubate for 1 hour at room temperature with gentle shaking.
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 0.5 hours 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.

Example

