



Mouse Eight-ANA ELISA Screen Kit

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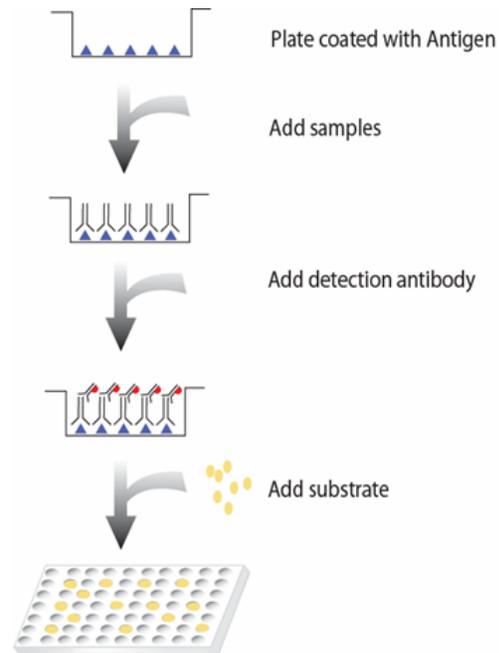
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Introduction

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-RNP (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.



Materials provided with the kit

- 8x12 96-well plate coated with 8 different antigens (4°C).
- Anti-mouse IgG antibody conjugated to HRP (4°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (4°C).
- Substrate (4°C).
- 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 1000 times of anti-mouse IgG antibody conjugated to HRP with 1X Diluent buffer.
- Avoid contact of Substrate and Stop Solution with sunlight or any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

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.

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 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-mouse 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. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
7. Add 100µl of Substrate to each well and incubate for 10-30 minutes.
8. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
9. Determine the optical density of each well with a

Mouse Eight-ANA ELISA Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	dsDNA											
B	SmD1											
C	U1-RNP											
D	SS-A/Ro											
E	SS-B/La											
F	Scl-70											
G	Jo-1											
H	CENP-B											