Signosis
Innovative Plate Assay Solutions

Anti-Jo-1 ELISA Kit

Catalog Number EA-5009 **(For Research Use Only)**

Introduction

Anti-Jo-1 antibody is a myositis specific autoantibody most commonly found in patients of Polymyositis (PM) and Dermatomyositis (DM). PM and DM are idiopathic inflammatory myopathies, characterized by proximal muscle weakness, elevated muscle enzyme activities and electromyographic and histological feature. This antibody is directed against the histidyl-tRNA synthetase which catalyses the binding of the histidine to its cognate tRNA during protein synthesis. Anti-Jo-1 antibody is predominantly found in 20-30% of PM patients and 60-70% of PM with interstitial pulmonary fibrosis. The antibody is also found in DM, although less frequently than in PM. , It is rare in children with PM or DM and in other connective tissue diseases. Moreover, the serum levels of anti-Jo-1 antibody strongly correlate with disease activity representing a good marker for disease monitoring.

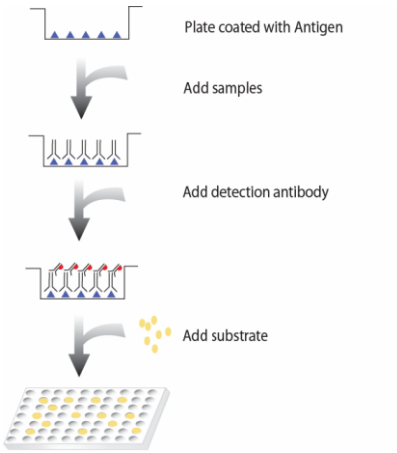

Diagram of ELISA

Principle of the assay

Anti-Jo-1 ELISA kit measures anti-Jo-1 antibodies in the serum. It is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes Jo-1 protein for immobilization on the microtiter wells and anti-mouse IgG antibodies conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two components, resulting in anti-Jo-1 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-Jo-1 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Materials provided with the kit

Component	Qty	Store at
8x12 96-well strip Plate coated with Jo-1	1	4°C
Anti-Human IgG antibody conjugated to HRP	10µL	4°C
Positive control	10µL	-20°C
1xDiluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	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-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. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
2.Add 100 µl of diluted samples or positive control (1:100 diluted with 1X Diluent Buffer) per well and incubate for 1 hour at room temperature with gentle shaking. *Note: We recommend having a blank condition. For the blank, add only 1x 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 7-30 minutes. *Note: Positive control will turn blue. Samples should be stopped when blue color begins to appear in blank.
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 microplate reader at 450 nm within 30 minutes.