Signosis
Innovative Plate Assay Solutions

Mouse Anti-IF ELISA Kit

Catalog Number EA-5213 **(For Research Use Only)**

Introduction

Intrinsic factor (IF) is a glycoprotein that is secreted by parietal cells in the stomach lining which binds to vitamin B12 in the small intestine and enables its absorption. In pernicious anemia, the body produces autoimmune antibodies against IF. The loss of IF inhibits the small intestine's ability to absorb vitamin B12, resulting in vitamin B12 deficiency.

Principle of the assay

Anti-IF ELISA kit measures anti-IF antibodies in the serum. It is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes IF 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-IF 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-IF is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

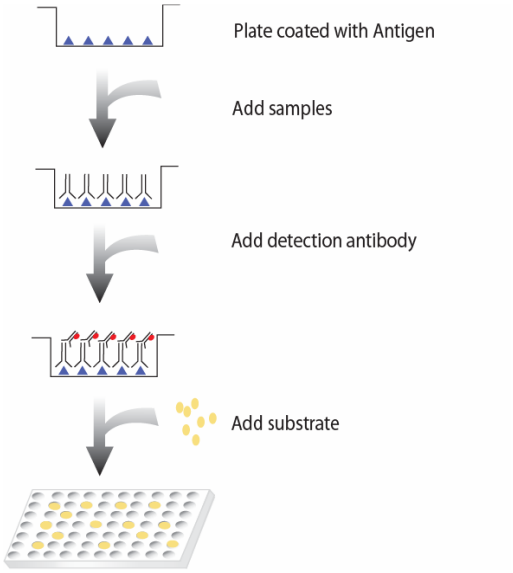

Diagram of ELISA

Materials provided with the kit

- 8x12 96-well plate coated with IF (4ºC).
- Anti-mouse IgG antibody conjugated to HRP (4ºC).
- Mouse IF Standard (4ºC).
- 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-mouse 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.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 g. Remove serum and assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Plasma

Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20° C. Avoid repeated freeze-thaw cycles.

Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
2. Standard Curve:
   - Add 200µl 1xDiluent Buffer to the 1st well on one strip
   - Add 100µl 1x Diluent Buffer to the rest of wells on the same strip
   - Add appropriate amount of mouse TPO standard (50 µg/ml) to 1st well as 1st dilution
   - Mix 1st dilution in 1st well and transfer 100µl from 1st to next well for next dilution. Perform six two-fold serial dilutions
   - 1xDiluent buffer serves as the zero standard or blank

Note: The first dilution starting from 250ng/ml is recommended.

3.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.

4. 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.
5. 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.
6. Repeat the aspiration/wash as in step 3.
7. Add 100µl of Substrate to each well and incubate for 5-15 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.