Signosis
Innovative Plate Assay Solutions

Mouse Galectin-3 ELISA Kit (Colorimetric)

Catalog Number EA-9009 **(For Research Use Only)**

Introduction

Galectin-3 is a member of the lectin family and contains a carbohydrate-recognition-binding domain that binds to β-galactoside. Due to its ability to bind to β-galactoside, galectin-3 plays an important role in cell-cell adhesion, cell-matrix interactions, macrophage activation, angiogenesis, metastasis, apoptosis. Given galectin-3's broad biological functionality, it has been demonstrated to be involved in a number of diseases including cancer, fibrosis, heart disease, and stroke. Overexpression of galectin-3 is commonly seen in cancerous conditions and can affect cancer cell growth and differentiation, chemoattraction, apoptosis, and metastasis. Because of this, galectin-3 is increasingly being used as a diagnostic marker for different cancers.

Principle of the assay

The wells of the plate are coated with capture antibody specific to galectin-3. In this assay, the test sample initially reacts with the solid phase capture antibody, resulting in galectin-3 being bound to the well. The wells are then washed to remove unbound proteins, and biotin-linked antibodies are added to bind to the immobilized galectin-3. After washing away the unbound antibodies, Streptavidin-HRP conjugate is added to form a complex with the antibody-bound galectin-3. After incubation, the wells are washed to remove unbound Streptavidin-HRP conjugate. TMB substrate is then added and forms a blue color when the HRP-linked antibodies are detected. The reaction is then terminated with Stop Solution, which changes the color from blue to yellow. The galectin-3 concentration in each well is directly proportional to its color intensity and can be quantified by measuring its optical density at 450 nm (OD450) in a microplate reader.

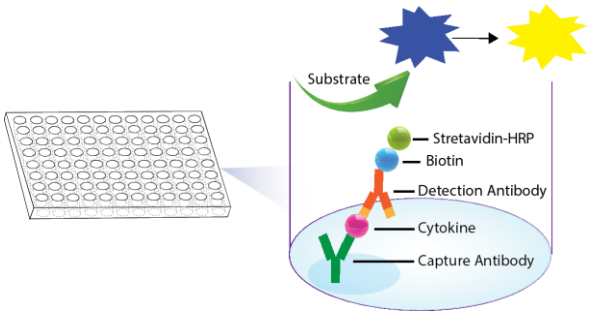

Diagram of Mouse Galectin-3 ELISA

Materials provided with the kit

Component	Qty	Store at
One clear plate coated with antibody against galectin-3	1	4°C
Biotin-labeled anti-galectin-3 antibody	200 µL	-20°C
Streptavidin-HRP conjugate	50 µL	4°C
1x Diluent buffer	40 mL	4°C
5x Assay wash buffer	40 mL	4°C
Substrate	10 mL	4°C
Stop solution	5 mL	4°C

Material required but not provided

- Microplate reader
- Distilled H2O

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  - 40 ml 5x Assay wash buffer
  - 160 ml ddH2O
- Dilute 50 times of biotin labeled antibody with 1X Diluent buffer.
  (AVOID FREEZE/THAW OF ANTIBODY MIX)
- Dilute 200 times of streptavidin-HRP with 1x Diluent buffer.

Sample preparation before starting experiment

- For **cell culture medium samples**, add 100 ul directly to the well or dilute 2-fold with 1X Diluent buffer.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol on Cell Lysate Buffer User Manual on our website.
- For **serum or plasma samples**, we recommend a 1:10 to 1:20 dilution with 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

Assay procedure

1. Take the plate from the aluminized bag. Seal the unused wells with a film.
2. Prepare 2.5 ml sample and add 100 µl of sample per well to one section and incubate for **2 hours** at room temperature with gentle shaking.
   Optional: If you want to have a blank reading, you can design one well as a blank well by adding diluent buffer instead of your sample.
3. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200 µl of 1x Assay wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against a pile of clean paper towels.
4. Add 100 µl of diluted biotin-labeled antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Add 100 µl substrate to each well and incubate for 30-40 minutes at least.

   Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped early after 5 minutes. Weaker signals should be incubated for 10-30 minutes.

9. Add 50 µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.