Signosis
Innovative Plate Assay Solutions

PPARγ ELISA Kit (Colorimetric)

Catalog Number TE-0009 **(For Research Use Only)**

Introduction

Peroxisome-proliferator-activated receptor gamma (PPARγ) is a ligand-activated transcription factor that plays an important role in the control of gene expression associated with a variety of physiological processes, particularly in metabolism and adipogenesis. With ligand binding, PPARγ forms a heterodimer with the retinoic X receptor, and binds to PPAR response elements in the promoter region of target genes, thus regulating their expression. Dysfunction of PPARγ leads to the pathological processes, such as metabolic diseases and cancer. Furthermore, modulation of receptor action in these diseases may be of therapeutic value. PPARγ has been demonstrated to be the target of the thiazolidinediones (TZDs), which are widely used to treat type 2 diabetes. Signosis has developed PPARγ ELISA, to help specific and sensitive measurement of the activation of PPARγ.

.Principle of the assay

PPARγ ELISA kit is high sensitive and specific assay with a simple and optimized procedure. The 96-well (8X12 strip) clear plate is pre-immobilized with the PPARγ consensus sequencing oligo. The activated PPARγ in nuclear extract or the whole cell lysate is added in the well and binds to the oligo. The activated PPARγ is detected with a specific antibody against PPARγ subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

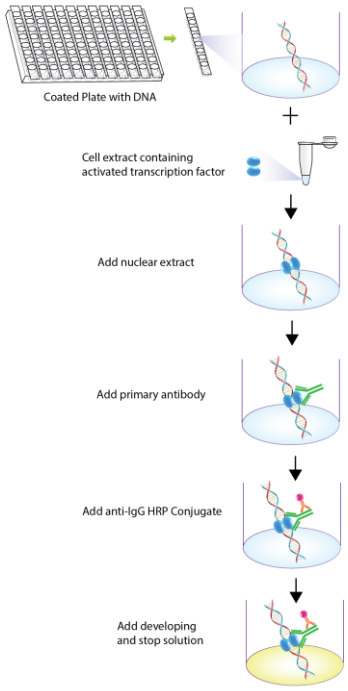

Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with PPARγ consensus oligo (4ºC).
- Antibody against PPARγ (4ºC).
- HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC).
- 1X Nuclear extract dilution buffer (-20ºC).
- PPARγ Positive control (-20ºC)
- 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
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 100 times of antibody against PPARγ with 1X Diluent buffer before use.
- Dilute 500 times of HRP conjugate secondary antibody with 1X Diluent buffer before use.

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Make TF binding mix
   30ul 2X TF binding buffer
   X Nuclear extract (2-10ug)
   X Nuclear extract dilution buffer
   Total 60ul
   For positive control, use 30ul of positive control without adding nuclear extract dilution buffer.
3. Add the mix on a well and incubate for 1 hour with gently shaking at room temperature.
4. Discard the contents and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 60µl of diluted antibody against PPARγ to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 60 µl of diluted HRP conjugate secondary antibody to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. Add 60µl of substrate to each well and incubate for 15-30 minutes.
10. Add 30µl of stop solution to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.