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PPARγ Ligand Screening/Characterization Assay Kit (Fluorometric)

12/16

(Catalog # K437-100, 100 assays; Store kit at -20°C)

I. Introduction:

The Peroxisome Proliferator Activated Receptor (PPAR) family of ligand-activated transcription factors consists of three subtypes encoded by separate genes: PPARα, PPARδ and PPARγ. Of these, PPARγ plays an important role in the regulation of fatty acid storage and glucose metabolism. The genes activated by PPARγ stimulate lipid uptake and adipogenesis by fat cells. Many endogenous molecules such as, polyunsaturated fatty acids like arachidonic acid and its metabolites, are known to bind and activate PPARγ. The binding of activating ligands to the ligand binding domain (LBD) of PPARγ promotes its heterodimerization with retinoic acid-like receptor (RXR), which results in the regulated expression of target genes involved in lipid metabolism. Such ligand-based activation of PPARγ may be responsible for inhibiting the growth of cultured human breast, gastric, lung, prostate and other cancer cell lines. In addition, the thiazolidinedione-based anti-diabetic drugs activate PPARγ with greater specificity than PPARα. BioVision's PPARγ Ligand Screening Assay Kit provides a single step fluorescence-based assay for screening potential PPARγ-specific ligands. The assay utilizes the ability of potential PPARγ-binding ligands to displace a fluorescent probe, which has a strong affinity for PPARγ Ligand Binding Domain, resulting in loss of fluorescence of the probe. The relative drop in the fluorescence, as a result of competitive binding of PPARγ ligand, can be correlated to the affinity (and hence IC_{50}) of the PPARγ candidate ligand. BioVision's PPARγ Ligand Screening Assay Kit is easy to use, faster and more convenient as compared to Fluorescence Polarization and TR-FRET-based screening methods. The assay kit can be used to identify and characterize PPARγ-specific ligands for therapeutic applications.

Probe + PPARγ $\xrightarrow{\textbf{- PPARγ Ligand}}$ High Fluorescence (Ex/Em = 375/460-470 nm)

Probe + PPARγ $\xrightarrow{\textbf{+ PPARγ Ligand}}$ Low Fluorescence (Ex/Em = 375/460-470 nm)

II. Applications:

- Screening of potential PPARγ binding ligands.

III. Kit Contents:

Components	K437-100	Cap Code	Part Number
PPARγ Assay Buffer	25 ml	WM	K437-100-1
PPARγ Assay Probe	10 µl	Red	K437-100-2
PPARγ (Human Recombinant)	2 x 250 µl	Brown	K437-100-3
PPARγ Ligand Control (100 mM in DMSO)	10 µl	Blue	K437-100-4
384-well Low Volume Black Plate	1 Plate	-	K437-100-5

IV. User Supplied Reagents and Equipment:

- DMSO, 384-well black plate.
- Multi-well spectroflurometer.

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the assay.

- **PPARγ Assay Buffer:** Bring to room temperature before use. Store at -20°C. Avoid prolonged storage of the PPARγ Assay Buffer at room temperature or 4°C.
- **Human PPARγ:** Store at -80°C. Avoid repeated freeze/thaw cycles. Each vial contains enough protein for 50 assays.
- **PPARγ Assay Probe and Ligand Control:** Store at -20°C. Bring to room temperature before use.

VI. PPARγ Ligand Screening Assay Protocol:

1. **PPARγ Assay probe preparation**: Dilute 5 µl of the PPARγ Assay Probe with 495 µl of DMSO. Mix well by light Vortexing. Use the probe immediately.
2. **Screening Compounds, Inhibitor Control & Blank Control Preparations:** Dissolve the test ligands in DMSO or other appropriate solvent. Use 1 µl of test ligand (Sample, **S**) or 1 µl DMSO (Solvent Control, **SC**) into empty well(s). For Ligand Control (**LC**), dilute 10X by adding 1 µl of PPARγ Ligand Control to 9 µl DMSO. Use 1 µl of 10x diluted PPARγ Ligand Control (in DMSO) into each well(s). In order to obtain IC_{50} values, different concentrations of test ligand and/or PPARγ Ligand Control should be tested.
3. **PPARγ Assay Mix:** Based on number of samples to be tested, prepare appropriate amount of PPARγ Assay Mix per well as below:

PPARγ Protein	5 µl
PPARγ Assay Probe (diluted)	1 µl
PPARγ Assay Buffer	18 µl
Total Volume	24 µl

Mix well by pipetting up and down. Incubate at RT for 5-10 min. Add 24 µl of PPARγ Assay Mix to each well containing test, solvent control and ligand control. Incubate at RT for 5 min before reading. Final reaction volume in each well shouldn't exceed 25 µl. Store unused PPARγ protein immediately at -80°C.

Notes:

a. If the test ligand is insoluble at high concentrations, precipitation might be observed during the assay. In that case, DMSO can be used up to 10% of final assay volume to increase the solubility of the test ligand in final assay solution.

4. **Measurement:** Measure the fluorescence intensity (Ex/Em = 375/460-470 nm) of the samples and the controls in an endpoint mode. The fluorescence signal is stable up to 1 h with minimum loss.
5. **Calculations**: Plot the % Relative Fluorescence (RFU, drop in the fluorescence intensity) and plot it against increasing concentration of the test ligand in the assay as given below; obtain IC_{50}.

$$\%\ \boldsymbol{Relative\ \ Fluorescence} = \frac{\boldsymbol{RFU\ (S)}}{\boldsymbol{RFU\ (SC)}} \times \mathbf{100}$$

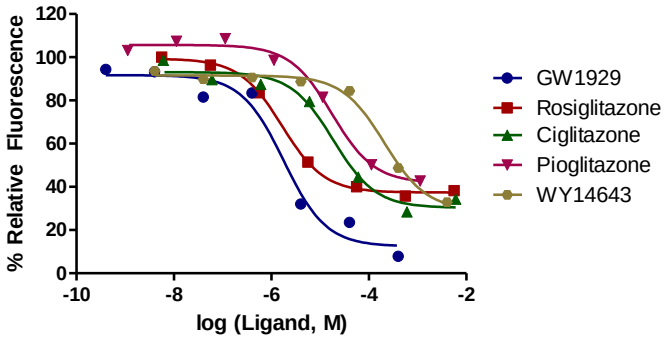

Figure: A variety of PPARγ-specific ligands (GW 1929, Rosiglitazone, Ciglitazone and Pioglitazone) and a PPARα-specific ligand (WY 14643) were tested using PPARγ Ligand Screening Assay Kit. Assays were performed following the kit protocol.

VII. RELATED PRODUCTS:

PPARγ (LBD) Human Recombinant (His-tagged) (7878)
PPAR gamma Antibody (3809)
PPARγ Antagonist, G3335 (1979-10,-25)
GW1929 (2057-5,-25)
Rosiglitazone (1559-5,-50,-100)
PPARɣ, Human, Recombinant (4371)
PPAR gamma Blocking Peptide (3809BP)
Ciglitazone (1695-5)
Pioglitazone (1877-5,-25,-100)
Troglitazone (1696-5)

FOR RESEARCH USE ONLY! Not to be used on humans.