ab284546– PPAR-gamma Ligand Screening/Characterization Assay Kit (Fluorometric)

For the screening of potential PPAR-gamma-specific ligands.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284546

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
384-Well Low Vol Black Plate/384-Well Low Volume Black Plate	1 unit	-20°C
PPARγ/PPAR-gamma (Human Recombinant)	2 x 250 µL	-20°C
PPARγ Assay Buffer/PPAR-gamma Assay Buffer	25 mL	-20°C
PPARγ Assay Probe/PPAR-gamma Assay Probe	10 µL	-20°C
PPARγ Ligand Control/PPAR-gamma Ligand Control (100 mM in DMSO)	10 µL	-20°C

Materials Required, Not Supplied

- DMSO
- Multi-well spectrofluorometer

Reagent Preparation

– Before using the kit, spin the tubes prior to opening.

PPARγ Assay Buffer/PPAR-gamma Assay Buffer: Bring to room temperature before use. Store at -20°C. Avoid prolonged storage of the PPARγ Assay Buffer/PPAR-gamma Assay Buffer at room temperature or 4°C

PPARγ/PPAR-gamma (Human Recombinant): Store at -80°C. Avoid repeated freeze/thaw cycles. Each vial contains enough protein for 50 assays.

PPARγ Assay Probe/PPAR-gamma Assay Probe and PPARγ Ligand Control/Ligand Control: Store at -20°C. Bring to room temperature before use.

Assay Protocol

PPARγ Assay Probe/PPAR-gamma Assay Probe preparation:

1. Dilute 5 µL of the PPARγ Assay Probe/PPAR-gamma Assay Probe with 495 µL of DMSO. Mix well by light Vortexing. Use the PPARγ Assay Probe/probe immediately.

Screening Compounds, Inhibitor Control, and Blank Control Preparations

1. 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).
2. For Ligand Control (LC), dilute 10X by adding 1 µL of PPARγ Ligand Control/PPAR-gamma Ligand Control to 9 µL DMSO.
3. Use 1 µL of 10x diluted PPARγ Ligand Control/PPAR-gamma Ligand Control (in DMSO) into each ligand control well(s). In order to obtain IC_{50} values, different concentrations of test ligand and/or PPARγ Ligand Control/PPAR-gamma Ligand Control should be tested.

PPARγ/PPAR-gamma Assay Mix Preparation

1. Based on number of samples to be tested, prepare appropriate amount of PPAR-gamma Assay Mix per well as below:

Item	PPAR-gamma Assay Mix
PPARγ/PPAR-gamma (Human Recombinant)	5 µL
PPARγ Assay Probe/PPAR-gamma Assay Probe (Diluted)	1 µL
PPARγ Assay Buffer/PPAR-gamma Assay Buffer	18 µL
Total Volume	24 µL

2. Mix well by pipetting up and down.
3. Incubate at room temperature for 5-10 minutes.
4. Add 24 µL of PPAR-gamma Assay Mix to each well containing test, solvent control and ligand control.
5. Incubate at room temperature for 5 minutes before reading.
6. Final reaction volume in each well shouldn't exceed 25 µL.
7. Store unused PPAR-gamma protein immediately at -80°C.

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 hour with minimum loss.

Calculation

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}.

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

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)