CREATIVE BIOMART®
Assay Kit

TR-FRET PPAR gamma Coactivator Assay Kit, rabbit

Product Information

Cat	
Kit-0976	
Common Name	
TR-FRET	
Cat.No.	Kit-0976

Description

The TR-FRET PPAR gamma Coactivator Assay Kit provides a sensitive and robust method for high-throughput screening of potential PPAR gamma ligands as agonists or antagonists of ligand-dependent coactivator recruitment. The kit uses a terbium (Tb)-labeled anti-GST antibody, a fluorescein-labeled coactivator peptide, and a Peroxisome Proliferator Activated Receptor (PPAR) gamma ligand-binding domain (PPAR gamma-LBD) that is tagged with glutathione-S-transferase (GST), in a homogenous mix-and-read assay format.

Agonist mode

To run the TR-FRET Peroxisome Proliferator Activated gamma Receptor Coactivator Assay in agonist mode (to identify agonist compounds), PPAR gamma-LBD is added to ligand test compounds followed by addition of a mixture of the fluorescein-coactivator peptide and Tb-anti-GST antibody. After an incubation period at room temperature, the TR-FRET 520: 495 emission ratio is calculated and used to determine the EC50 from a dose response curve of the compound. Based on the biology of the PPAR gamma-coactivator peptide interaction, this ligand EC50 is a composite value representing the amount of ligand required to bind to receptor, effect a conformational change, and recruit coactivator peptide.

Antagonist mode

When the TR-FRET Peroxisome Proliferator Activated gamma Receptor Coactivator Assay is run in antagonist mode (to identify antagonist compounds), PPAR gamma-LBD is added to ligand test compounds followed by addition of a mixture of agonist, fluores-cein-coactivator peptide, and Tb-anti-GST antibody. The concentration of agonist used in this mode is the EC80 concentration as determined by first running the assay in agonist mode.

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Applications

Nuclear Receptor Assay

Storage

Tb-anti-GST Antibody (Rabbit): Store at -20 °C
PPAR gamma-LBD, GST: Store at -80 °C
Fluoroscein Coactivator Peptide: Store at -20 °C
TR-FRET Coregulatore Buffer F: Store at 4 °C
1M DTT: Store at -20 °C or -80 °C

Size

800 x 20 µL assays

Kit Components

The TR-FRET PPARγ Coactivator Assay Kit contains PPAR gamma-LBD (GST) protein, fluorescently labeled TRAP220/ DRIP-2 coactivator peptide, Tb-anti-GST antibody, and buffers. Store components as indicated in the assay protocol (-80°C, -20°C, or +4°C).

Materials Required but Not Supplied

Microplate Reader

Detection method Fluorescent

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