CREATIVE BIOMART®
Assay Kit

TR-FRET ER alpha Coactivator Assay Kit

Product Information

Cat

Kit-0951

Common Name

TR-FRET

Cat.No. Kit-0951

Description

The TR-FRET ER alpha Coactivator Assay Kit provides a sensitive and robust method for high-throughput screening of potential estrogen receptor (ER) alpha ligands as agonists or antagonists of ligand-dependent coactivator recruitment. The kit uses an ER alpha ligand-binding domain (ER alpha-LBD) tagged with glutathione-S-transferase (GST), a terbium (Tb)-labeled anti-GST antibody, and a fluorescein-labeled coactivator peptide in a homogenous mix-and-read assay format.
Agonist Mode
To run the LanthaScreen TR-FRET ER alpha Coactivator Assay in agonist mode (to identify agonist compounds), ER alpha-LBD is added to ligand test compounds, followed by the addition of a mixture of fluorescein-coactivator peptide and Tb-anti-GST antibody. After incubation at room temperature, the TR-FRET 520: 495 nm emission ratio is calculated and used to determine the EC50 from a dose response curve of the compound. Based on the biology of the ER alpha-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
To run the LanthaScreen TR-FRET ER alpha Coactivator Assay in antagonist mode (to identify antagonist compounds), ER alpha-LBD is added to ligand test compounds, followed by addition of a mixture of agonist, fluorescein-coactivator peptide, and terbium (Tb)-labeled 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.

Applications

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Assay Kit

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Co-Factor Interaction Assay, Nuclear Receptor Assay

Storage

1 tube ER alpha Ligand Binding Domain Recombinant Protein (store at -68 to -85°C)
1 tube of Peptide (store at -5 to -30°C)
2 bottles TR-FRET Buffer (store at -5 to -30°C)
1 tube Tb-Anti-GST Ab (store at -5 to -30°C)
1 tube of DTT (store at -5 to -30°C)

Size

800 x 20 µL assays

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