Description

The FXR (Farnesoid X Receptor) Agonist Assay Kit (TR-FRET) is designed to measure binding of the FXR and SRC-1 (Steroid Receptor Coactivator 1) for screening FXR agonists using TR-FRET technology. With this homogeneous kit, the FXR agonist assay can be performed by adding all materials, including the detection reagents, to the wells and incubating the plate for 2-hours at room temperature followed by reading the TR-FRET signal.

Background

Nonalcoholic fatty liver disease (NAFLD) is a characterized by the aberrant accumulation of triglycerides in hepatocytes, even in the absence of significant alcohol consumption or viral infection. The FXR is a nuclear receptor that maintains bile acid homeostasis, and aberrant bile acid signaling via activation of FXR contributes liver disease. Several small molecule agonists have been developed and tested in clinical trials for NASH (Non-Alcoholic SteatoHepatitis), and the FXR pathway is also a molecular target in the search for novel cholesterol lowering agents.

Applications

Useful for screening small molecular FXR agonists for drug discovery and HTS applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100410	FXR, GST-tag	3 µg	-80°C
	SRC-1, Biotin labeled (10 µM in DMSO)	20 µl	-80°C
	CDCA (10 mM in DMSO)	50 µl	-20°C
	FXR Binding Buffer	20 ml	-20°C
	Tb donor	2 X 10 µl	-20°C
	Dye labeled acceptor	2 X 10 µl	-20°C
79969	384-well plate, white, nonbinding	1 EA	Room Temp.

Materials Required but Not Supplied

Name	Catalog #
Adjustable micropipettor and sterile tips	
Microplate centrifuge	

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

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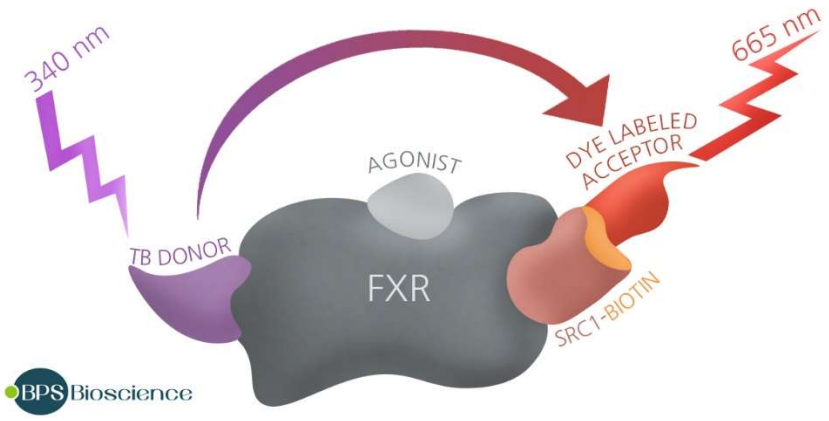
Assay Principle



Upon binding of the agonist to the FXR, SRC-1-Biotin and FXR form a heterodimer, resulting in energy transfer from Tb donor to the dye labeled acceptor.

Assay Protocol

All samples and controls should be tested in duplicate.

Preparing Your Reagents

1. Thaw FXR, SRC-1-Biotin and FXR Binding Buffer.
2. Dilute the test agonists into distilled water at 10X testing concentration. (*e.g*. To test at 100 nM, prepare a 10 µM solution of the test compound in DMSO. Mix 10 µl of the 10 µM solution and 90 µl of distilled water to create a 1 µM testing test compound in 10% DMSO(aqueous) The final concentration of DMSO in the assay must be no more than 1%. For the "Negative" control, prepare 10% DMSO(aqueous) solution (*i.e*. Inhibitor buffer) by mixing 10 µl DMSO and 90 µl distilled water. For the "Positive" control, prepare 500 µM CDCA solution in 10% DMSO(aqueous), i.e. CDCA final concentration is 50 µM.

Component	Negative Control	Positive Control	Test Inhibitor
FXR, GST-tag (~ 0.4 µg/ml)	3 µl	3 µl	3 µl
SRC-1, Biotin labeled (40 nM)	5 µl	5 µl	5 µl
Test Agonist	-	-	2 µl
CDCA (500 µM) in 10% DMSO (aqueous)	-	2 µl	-
10% DMSO (aqueous)	2 µl	-	-
	Centrifugation		
Diluted Tb-donor	5 µl	5 µl	5 µl
Diluted Labeled acceptor	5 µl	5 µl	5 µl
	Centrifugation		
Total	20 µl	20 µl	20 µl

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3. Dilute the FXR in the FXR Binding Buffer at 0.4 µg/ml (~ 6.6 nM)
   Protein is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme
4. Dilute SRC-1-Biotin in the FXR Binding Buffer at 40 nM. The remaining SRC-1-Biotin can be stored in aliquots at -80°C
5. Add 3 µl of the diluted FXR to all wells.
6. Add 5 µl of the diluted SRC-1-Biotin to all wells.
7. Add 2 µl of the test inhibitor prepared in Step 2 (For the "Negative" and "Blank" controls, add 2 µl of 10% aqueous DMSO solution. For the "Positive" control, add 2 µl of 500 µM CDCA in 10% DMSO(aqueous).
8. Briefly centrifuge the plate to ensure all components are mixed.
9. Thaw Tb-donor and Dye labeled acceptor on ice and dilute them 100-fold in the FXR Binding Buffer. Prepare only sufficient quantities needed for the assay. The remaining reagents can be stored at -20°C.
10. Add 5 µl of the diluted Tb-donor to the wells. Add 5 µl of the diluted Dye labeled acceptor to the wells (Alternatively, diluted Tb-donor and Dye labeled acceptor can be mixed at 1:1 ratio and add 10 µl to the wells.)
11. Briefly centrifuge the plate to ensure all components are mixed, and incubate the plate for 2 hours at room temperature

Protect your samples from direct exposure to light.

12. Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	340±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS:

Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

TR-FRET ratio of the Negative control (no agonist) can be set at 0% agonistic effect. For the 100% agonistic effect, either the TR-FRET ratio from the 50 µM CDCA sample or the highest TR-FRET ratio from the testing compound can be used.

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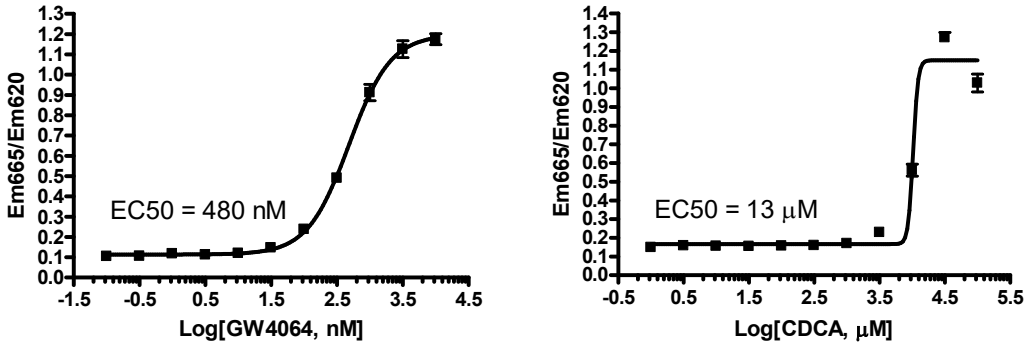
Example Results



FXR agonistic effect of GW4064 and CDCA were measured using FXR Agonist Assay Kit (TR-FRET) (BPS Bioscience, #78131). For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General considerations

"Blank" Control: The "Blank" control is not required but can be set up if needed. Add all the components as in the negative control except SRC-1-Biotin. Instead of SRC-1-Biotin, add 5 µl FXR Binding Buffer.

Trouble Shooting Guide

Problem	Possible Causes	Recommended Solutions
TR-FRET signal of positive control reaction is same as "Blank" value.	FXR has lost activity	The protein can be damaged by repeated freeze/thaw cycles. Use fresh FXR, BPS Bioscience #100410. Store enzyme in single use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Tb Donor beads or dye labeled acceptor beads fail to show significant signal.	Reorder Tb beads or dye-labeled acceptor beads from Perkin Elmer. Different lots have shown wide variation in activity.
	Incorrect settings on instruments	Refer to instrument instructions for correct settings to increase sensitivity of light detection.
TR-FRET signal is erratic or varies widely among wells	Inaccurate pipetting/ technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.

Visit. bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

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References

1. Angela D., *et al.* Targeting Bile Acid Receptors: Discovery of a Potent and Selective Farnesoid X Receptor Agonist as a New Lead in the Pharmacological Approach to Liver Diseases. *Front Pharmacol.* 2017; **8(162):**1-13.
2. Sills M., *et al.* A Comparison of ALPHAScreen, TR-FRET, and TRF as Assay Methods for FXR Nuclear Receptors. *J Biomol Screen*. 2002; **7(1):**3-10

Related Products

Products	*Catalog #*	*Size*
FXR, GST-Tag	100456	2 vials
LDLR, FLAG-tag	71205	50 µg
PCSK9, His-tag	71204-2	50 µg

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