PolarScreen™ Nuclear Receptor Competitor Assays - Universal Protocol

Shipping: Varies
Storage: Varies
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For Research Use Only. Not for use in diagnostic procedures.

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Kit Contents

PolarScreen™ Nuclear Receptor Competitor Assay kits include all the reagents needed to perform the assay:

- Nuclear receptor (NR) protein
- Fluormone™ Tracer
- NR-specific buffer
- DTT (if required)

For a detailed list of kit components, refer to the Product Information Sheet supplied with the kit or visit **www.lifetechnologies.com** and search using the assay part number.

Kit Handling

PolarScreen™ Nuclear Receptor Competitor Assay kits are shipped on dry ice. Upon receipt, store components as indicated on the Product Information Sheet supplied with the kit.

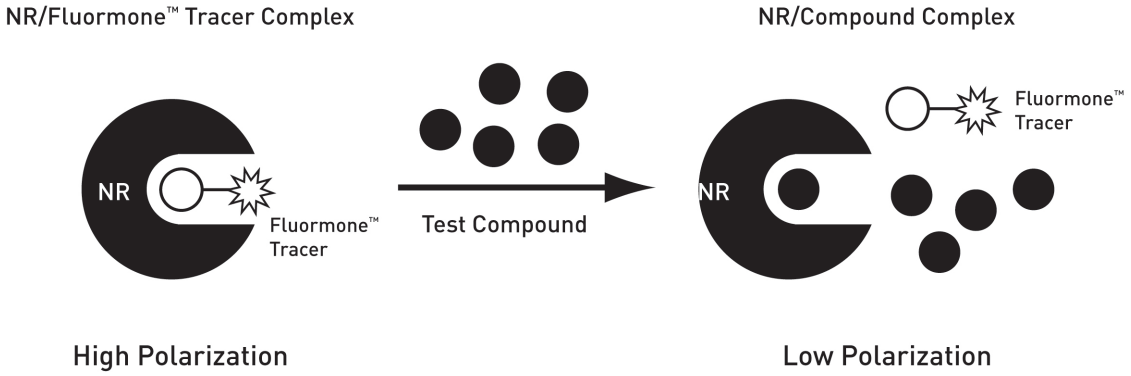
Materials Required but Not Provided

Material	Description	Source	Comments
Fluorescence plate reader	Plate reader capable of reading fluorescence polarization (FP)	Various	For more information, contact Technical Support at **drugdiscoverytech@lifetech.com**.
Fluorescence polarization filters green	Excitation/emission wavelengths 485/535 nm, bandwidths of 25/20 nm	Instrument Vendor/ Various	Check instrument manual.
Fluorescence polarization filters red	Excitation/emission wavelengths 535/590 nm, bandwidths of 25/20 nm	Instrument Vendor/ Various	Check instrument manual.
Pipetting devices	1–1000 µL volumes	Various	Repeater and/or multi-channel pipettes are preferred.
DMSO Serial Dilution Plate	384-deep well, uncoated, polypropylene plates that can accommodate 240 µL per well; plate must be tolerant of 100% DMSO	Corning, Cat. no. 3363, or similar	Used for ligand serial dilutions in 100% DMSO.
Intermediate Dilution Plate	384-deep well	Various	
Assay Plate	Black 384-well plate See the Product Information Sheet for specific assay plate recommendations.	Various	Clear bottom and white plates are unsuitable for use.
DMSO	High purity	Sigma, Cat. no. 41647	Used to perform compound dilutions.
Control ligand	Known agonist or antagonist for a specific NR	Various	See Product Information Sheet.
FP One-Step Reference Kit	Used to determine if the instrument is measuring polarization values accurately and to set G-factor	Life Technologies, Cat. no. P3088	Recommended for first time users.

Overview

The PolarScreen™ Nuclear Receptor Fluorescence Polarization (FP) Competitor Assay provides a sensitive and efficient method for non-radioactive, high-throughput screening of potential nuclear receptor ligands. The kit uses the nuclear receptor protein and a novel, tight-binding, selective fluorescent ligand, Fluormone™ Tracer, in a homogenous, mix-and-read assay format. The assay is optimized to bind 80% of the Fluormone™ Tracer for optimal assay window without right shifting IC_{50} values.

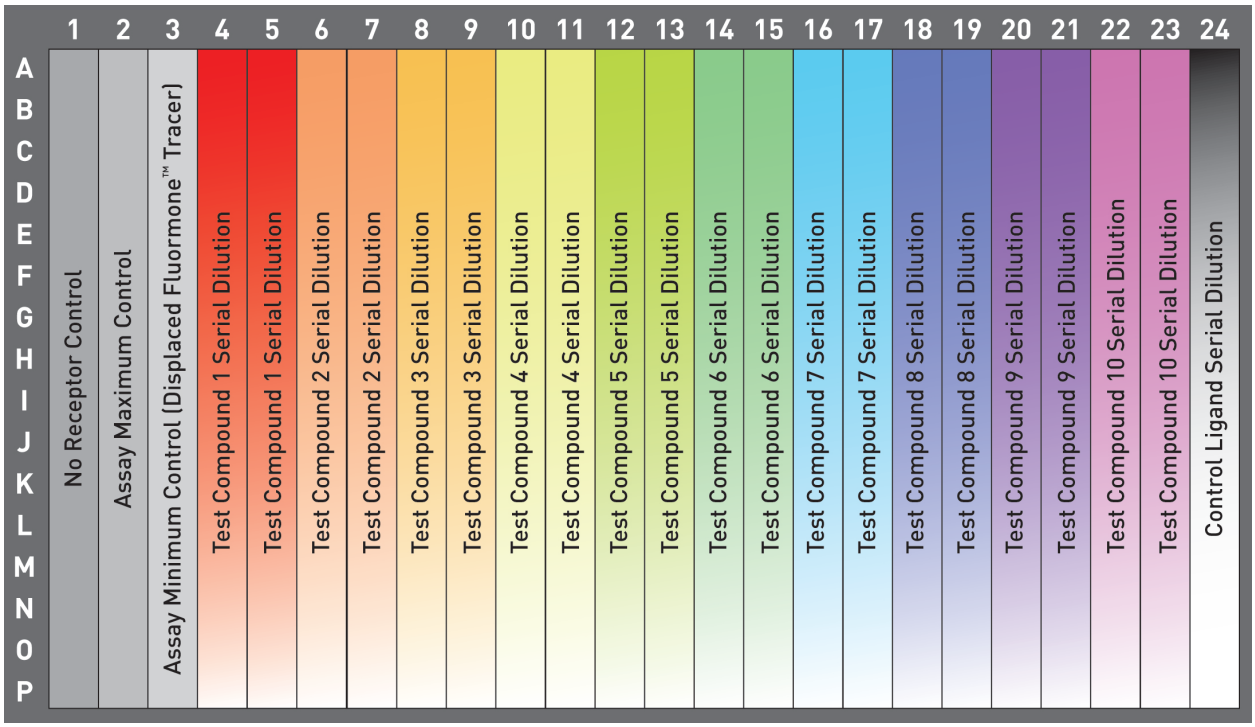
The nuclear receptor (NR) protein and Fluormone™ Tracer form a NR/Fluormone™ Tracer complex, resulting in a high polarization value. Compounds that displace the Fluormone™ Tracer from the NR/Fluormone™ Tracer complex cause a decrease in polarization. Displaced Fluormone™ Tracer tumbles rapidly, resulting in a low polarization value (see illustration below). The polarization remains high in the presence of compounds which do not displace the Fluormone™ Tracer from the complex. The shift in polarization value in the presence of test compounds is used to determine relative affinity of test compounds for the NR.



Assay Controls

Control	Reagent	Purpose
No Receptor Control (Free Fluormone™ Tracer Control)	Fluormone™ Tracer	Provides baseline absolute minimum mP (polarization) value and serves as a troubleshooting reference when there is no difference between the Maximum and Minimum mP Assay Control (see below).
Assay Maximum mP Control	NR/Fluormone™ Complex	Provides absolute maximum mP signal for assay.
Assay Minimum mP Control (Displaced Fluormone™ Tracer)	• NR/Fluormone™ Complex • Saturating control ligand	Provides **bottom** baseline for assay, including non-specific ligand binding.

Assay Plate Layout



Preparing and Delivering Reagents

The assay described herein can accommodate up to 10-different test compounds in a single plate. Prepare the compounds and set up the plate as completely as possible before preparing the NR for addition.

- The total assay volume is 20 µL per well. The reagent volumes given below assume 20 µL assay volume.
- The solvent is typically DMSO. The protocol below refers to DMSO when solvent is mentioned.
- Typically, there are 3 plates used in the assay:
    1) DMSO serial dilution plate (DMSO tolerant)
    2) Intermediate dilution plate (384-deep well)
    3) Assay plate (as recommended in the Product Information Sheet)

Prepare Complete NR Buffer

1. Prepare complete NR buffer sufficient to support 10 µL buffer per well, plus 100 µL buffer per compound, and an additional 10% for pipetting errors.
2. Some NRs require the addition of DTT to the assay buffer; refer to the NR Product Information Sheet for more information. In either case, the assay buffer is referred to as "complete NR buffer" in the instructions below.

Note: Prepare complete NR buffer sufficient for assays to be performed in a single day. Prolonged storage of the complete NR Buffer with DTT results in oxidation of the DTT and sub-optimal assay performance.

Prepare Complete NR Buffer with 2X DMSO

1. Prepare 10 µL of complete NR buffer per well using 2% DMSO; typically 32 wells per 384-well plate are used.
2. Deliver 10 µL of complete NR buffer to each well in columns 1 and 2 of the assay plate.

Prepare Control Ligand Stock, transfer to the assay plate

Note: Concentrated control ligand stock (100X) is typically at 1 mM. Refer to the NR Product Information Sheet for NR-specific control ligand.

Note: Use 16 wells of **Assay Minimum Control** per plate, which requires ~200 µL of control ligand per plate.

1. Prepare concentrated stock of control ligand at 100X in DMSO, typically 1 mM.
   a. Determine the mass of the ligand to 0.000 mg.
   b. Calculate the correct volume of DMSO to add to the weighed ligand to bring the concentration to 1 mM.
2. Prepare **Assay Minimum Control** by diluting the concentrated 1 mM stock to 2X using the complete NR buffer.
3. Deliver 10 µL of the 2X Assay Minimum Control to each well in column 3 of the assay plate.

Important: Differences or inaccuracies in the methods used to prepare the concentrated stock solution of the control ligand account for the majority of the differences in IC_{50} values observed in different laboratories.

Prepare the Test Compound(s), transfer to the assay plate

Note: We recommend preparing a 16-point, 3-fold dilution series for each test compound. This method leads to a maximum final compound concentration of 10,000 nM.

Note: Treat the control ligand as a test compound, and prepare a 16-point, 3-fold dilution series. We recommend at least one column of titrated control ligand per assay plate (see **Assay Plate Layout**, column 24, on page 4).

Note: One assay plate supports up to 10 unique compounds.

1. Prepare concentrated stocks of test compounds at 100X in DMSO. Typical 100X concentration is 1 mM.
   a. Determine the mass of the ligand to 0.000 mg.
   b. Calculate the correct volume of DMSO to add to the weighed ligand to bring the concentration to 1 mM.
2. Prepare a 16-point serial dilution of each test compound at 100X in 100% DMSO in the DMSO tolerant plate. For more details, see **Additional Instructions, Preparing 100X DMSO serial dilution plate**, page 6.
3. Dilute each concentration of the 16-point serial dilution at 100X to 2X in the intermediate dilution plate using complete NR buffer. For more details, see **Additional Instructions, Preparing 2X intermediate dilution plate**, page 6.
4. Transfer 10 µL of the 2X compound intermediate concentration to duplicate columns of the assay plate, columns 4–24. For more details, see **Additional Instructions, Transferring 2X intermediate dilution of test compounds to assay plate**, page 6.

Important: Dilutions performed in 100% DMSO facilitate compound solubility during the serial dilutions, which is important for obtaining consistent IC_{50} values.

Note: Use a 100% DMSO tolerant plate (see **Materials Required but Not Provided**, page 2).

Additional instructions for preparing plates

Preparing 100X DMSO serial dilution plate

Note: Prepare a 16-point 100X DMSO dilution series of each test compound in a 384-well polypropylene plate (DMSO tolerant) using 100% DMSO.

Note: Dilution series are prepared down one column of the DMSO serial dilution plate. One column is required for each test compound.

1. Add 20 µL of 100% DMSO to wells B1–P1 in a 384-well plate.
2. Add 30 µL of 100X compound stock solution to well A1 of the same 384-well plate.
3. Transfer 10 µL from well A1 to well B1 and mix several times by pipetting up and down.
4. Transfer 10 µL from well B1 to well C1 and mix several times by pipetting up and down.
5. Repeat this process through well P1 and for all test compounds. 100X test compound concentrations are 1000 µM, 333 µM, 111 µM etc.

Preparing 2X intermediate dilution plate

Note: Dilute each 100X test compound serial dilution to 2X using the complete NR buffer.

Note: Intermediate dilution series occurs down one column of the 2X intermediate dilution plate. One column is required for each test compound.

1. Add 98 µL of the complete NR buffer to the required number of columns in the intermediate dilution plate.
2. Transfer 2 µL (minimum recommended volume for transfer) of the 100X DMSO test compound dilution series from each well of column 1 of the 100X DMSO serial dilution plate to each well of column 1 of the 2X intermediate dilution plate. Mix well by pipetting up and down.
3. Repeat for all test compounds.

Transferring 2X intermediate dilution of test compounds to assay plate

Note: Two columns required per test compound

1. Transfer 10 µL of the 2X intermediate dilution from column 1 of the 2X intermediate dilution plate to columns 4 and 5 of the assay plate. This provides duplicates for each concentration.
2. Transfer the required volume of the 2X intermediate dilution from column 2 of the 2X intermediate dilution plate to columns 6 and 7 of the assay plate.
3. Repeat for all test compounds.

Prepare 2X Fluormone™ Tracer (for the no receptor control), transfer to the assay plate

Note: Refer to the NR product information sheet for the required final (1X) concentration of Fluormone™ Tracer.

1. Prepare 2X Fluormone™ Tracer using the complete NR buffer.
2. Add 10 µL of 2X Fluormone™ Tracer to each well of column 1 of the assay plate.

Prepare 2X NR/Fluormone™ Complex or 4X Fluormone™ Tracer and 4X NR

Note: **Only certain NRs and Fluormone™ Tracers can be pre-mixed.** For NRs that can be pre-mixed, prepare the 2X NR/Fluormone™ Complex (see **Assays with pre-mix**, below); for NRs that cannot be pre-mixed, prepare the 4X Fluormone™ Tracer and 4X NR separately (see **Assays without pre-mix**, page 8). Refer to the table below for our recommendation.

Assay*	Cat. no.	Pre-mix allowed	Pre-mix not recommended
PolarScreen™ AR Competitor Assay, Green	A15880	X	
PolarScreen™ AR Competitor Assay, Red	A15881		X
PolarScreen™ ER Alpha Competitor Assay, Green	A15883 A15882	X	
PolarScreen™ ER Alpha Competitor Kit, Red	A15884	X	
PolarScreen™ ER Beta Competitor Assay, Green	A15890 A15889	X	
PolarScreen™ ER Beta Competitor Assay, Red	A15891	X	
PolarScreen™ GR Competitor Assay, Green	A15897		X
PolarScreen™ GR Competitor Assay, Red	A15898		X
PolarScreen™ PPARγ Competitor Assay, Green	PV6136	X	
PolarScreen™ PR Competitor Assay, Green	A15905	X	
PolarScreen™ PR Competitor Assay, Red	A15906	X	
PolarScreen™ VDR Competitor Assay, Red	A15907	X	

*Assay availability subject to change without notice; for most up-to-date offerings, refer to **www.lifetechnologies.com** or contact Technical Support at 760-603-7200, extension 40266 or 800-955-6288, extension 40266, or e-mail **drugdiscoverytech@lifetech.com**.

Assay Protocol

Note: For NRs that can be pre-mixed, follow the 2X NR/Fluormone™ Complex method, and for those that cannot be pre-mixed, prepare the 4X Fluormone™ Tracer and 4X NR separately.

Assays with pre-mix

Prepare and deliver 2X NR/Fluormone™ Complex

Note: Refer to the Product Information Sheet included with the product for final assay concentrations of NR and the Fluormone™ Tracer.

1. Prepare a 2X solution of NR/Fluormone™ Complex in complete NR buffer.
2. Add 10 µL of the 2X NR/Fluormone™ Complex to columns 2–24 of the assay plate.

Assays without pre-mix

Prepare and deliver 4X Fluormone™ Tracer solution

Note: Refer to the NR Product Information Sheet included with the product for final 1X assay concentration of Fluormone™ Tracer.

1. Prepare a 4X solution of Fluormone™ Tracer solution in complete NR buffer.
2. Add 5 µL of the 4X Fluormone™ Tracer solution to columns 2–24 of the assay plate.

Prepare and deliver 4X NR

Note: Refer to the NR Product Information Sheet included with the product for final 1X assay concentration of NR.

1. Prepare a 4X solution of NR in complete NR buffer.
2. Add 5 µL of the 4X NR solution to columns 2–24 of the assay plate.

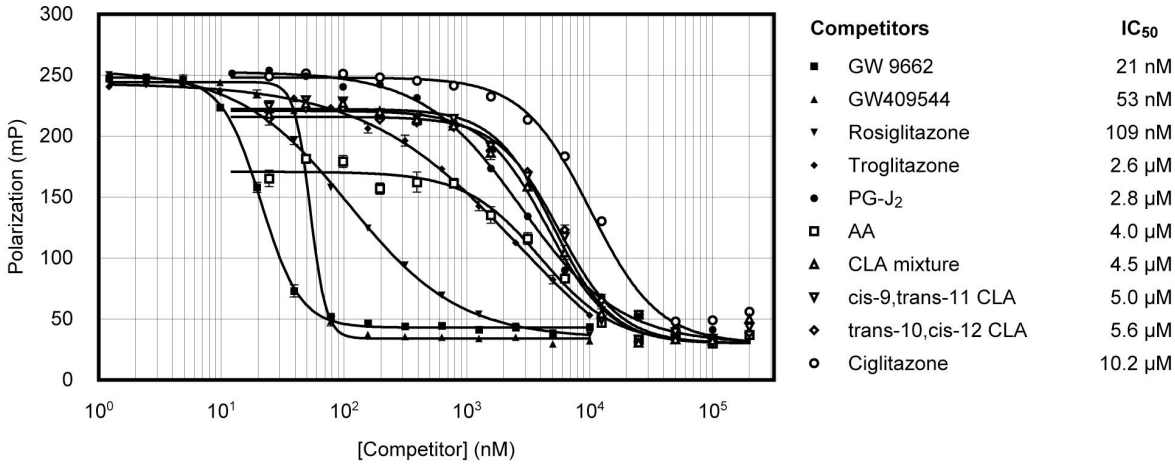
Process the assay plate

1. Mix the plate.
2. Cover the plate to protect the reagents from light.
3. Incubate the plate at room temperature for at least 2 hours. Refer to the Product Information Sheet included with the product for target specific information.
4. Measure fluorescence polarization value (mP) of each well on a fluorescence polarization plate reader.

Results and Discussion

Below is an example of competition data for PPARγ generated in a 384-well plate. Polarization values are plotted against the concentration of test compound. The concentration of the test compound that results in a half-maximal shift in polarization value equals the IC_{50} of the test compound, which is a measure of the relative affinity of the test compound for the PPARγ ligand binding domain. Error bars represent one standard deviation from the mean of triplicate reaction wells in a competition assay incubated for 2 hours before reading.

In the graph below, mP was plotted on the y-axis and the log[compound] (nM) was plotted on the x-axis. Curve fitting was performed using GraphPad Prism™ 4.0 software.



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