PolarScreen™ PPAR Gamma Competitor Assay, Green

Catalog no. PV6136 **Shipping:** Dry ice **Storage:** Varies

Publication no. MAN0009991 **Revision** A.0

Overview

PolarScreen™ Peroxisome Proliferator-activated Receptor Gamma (PPAR Gamma) Competitor Assay, Green is a binding assay for determining the IC_{50} values of compounds that bind the PPAR Gamma-LBD (GST). PPAR Gamma-LBD (GST) (Part no. PV4546) is supplied as 50 µg total protein and contains sufficient active PPAR Gamma-LBD (GST) to run the PPAR Gamma Competitor Assay, Green. The PV6136 kit contains enough reagents to perform the assay in up to 800 wells at 20 µL total assay volume when using the concentrations described on the lot-specific Certificate of Analysis (CoA).

Component	Composition	Storage	PV6136	
			Amount	Part no.
PPAR Gamma-LBD (GST)[1,4]	Buffer: 50 mM Tris-HCl (pH 8.0), 150 mM KCl, 2 mM DTT, 0.5 mM EDTA, 0.5% Big CHAP, 20% glycerol	–80°C	50 µg (total protein)	PV4546
Fluormone PPAR Gamma Green[2-3] (Fluormone Tracer)	1800 nM in 90% methanol/ 10% water	–20°C	100 µL	PV3356
PPAR Gamma Green Screening Buffer	Proprietary Buffer (pH 7.4), 0% glycerol	20°C to 30°C	2 x 20 mL	PV3358
DTT Solution	1 M DTT in water	–20°C	1 mL	P2325

[1-4]Notes begin on page 3.

Note: PPAR Gamma-LBD (GST) may aggregate with rough handling. Do not vortex. Do not expose PPAR Gamma-LBD (GST) to more than 5 freeze-thaw cycles. Once thawed, PPAR Gamma-LBD (GST) must remain on ice.

FAST FACTS

- For more detailed instruction on running a PolarScreen™ Nuclear Receptor Competitor Assay, go to **www.lifetechnologies.com**, search using the assay catalog number, and view **PolarScreen Nuclear Receptor Competitor Assays - Universal Protocol**
- For information on our Nuclear Receptor Portfolio, visit **www.lifetechnologies.com/nuclearreceptor.**
- We recommend using a low volume, black, round bottom, non-treated 384-well plate, (Corning®, Cat. no. 4511).
- We recommend a PPAR Gamma ligand, such as Rosiglitazone (Cayman Chemical®, Cat. no. 71740), as the control ligand.
- The Kd of the Fluormone PPAR Gamma Green with PPAR Gamma-LBD (GST) equals 32 ± 15 nM (based active receptor).
- Complete PPAR Gamma Green Screening Buffer with DTT must be used the day it is prepared. Add 5 µL I M DTT (P2325) per 1 mL of PPAR Gamma Screening Buffer (PV3358).
- Solvent tolerance, 1% DMSO final preferred, up to 3% DMSO, 8% MeOH and 5% EtOH may be used.
- Incubate assays at room temperature for two hours and read within 20 hours of mixing reagents. Use consistent time.

For Research Use Only. Not for use in diagnostic procedures.

Final assay conditions

Reagent	1X Final assay concentration
PPAR Gamma-LBD (GST)	See lot specific CoA[4]
Fluormone PPAR Gamma Green	9 nM

[4] We have observed that the optimal concentration of the nuclear receptor can be instrument dependent. See note 4, page 3, for additional details.

Quick start protocol

Note: Do not vortex PPAR Gamma-LBD (GST)/Fluormone Complex. The complex may be stored for up to 8 hours on ice (mP increases 15 mP from t = 0 to t = 8 hours) or at room temperature (mP increases 45 mP from t = 0 to t = 8 hours) with no significant affect on IC_{50} values.

Reagent volumes

The table below summarizes the reagent amounts required for performing the PolarScreen™ PPAR Gamma Competitor Assay, Green and the associated controls at 20 µL total assay volume.

Component	Assay	Controls		
	Test Compound	No Receptor Control (Free Fluormone Tracer Control)	Maximum mP Control	Minimum mP Control (Displaced Fluormone Tracer)
2X Saturating Rosiglitazone (20 µM)	—	—	—	10 µL
2X Test Compound (single points or titrations)	10 µL	—	—	—
2X PPAR Gamma-LBD (GST)/Fluormone PPAR Gamma Green Complex	10 µL	—	10 µL	10 µL
2X Fluormone PPAR Gamma Green	—	10 µL	—	—
Complete PPAR Gamma Green Screening Buffer with 2X DMSO (or other solvent)*	—	10 µL	10 µL	—

*The concentration of DMSO (or other solvent) in each well must be constant.

Note: Assay window, delta mP (ΔmP), is the difference between the ***Maximum mP Control*** and ***Minimum mP Control*** of displaced Fluormone Tracer; see table above.

Perform the assay

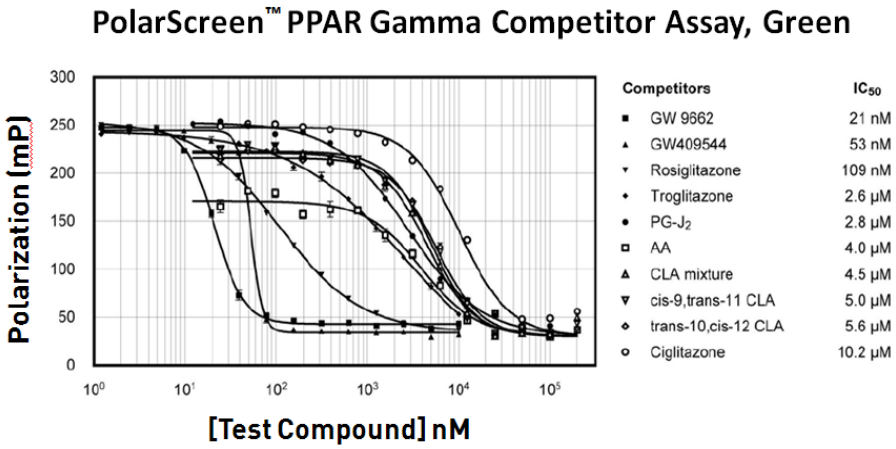
Note: Refer to the **PolarScreen™ Nuclear Receptor Competitor Assays - Universal Protocol** at **www.lifetechnologies.com** for assay plate layout and for detailed instructions on preparing and delivering the reagents.

Add the reagents listed in the table above into the appropriate wells of the assay plate.

1. Mix the assay plate.
2. Cover plate to protect reagents from light.
3. Incubate plate at room temperature for at least 2 hours.
4. Measure fluorescence polarization value (mP) of each well on a fluorescence polarization plate reader **wi**thin 20 hours of mixing the reagents.

Example data

An example of competitive binding data generated using the PolarScreen™ PPAR Gamma Competitor Assay, Green is shown below. Polarization values are plotted against the concentration of test compound. Data were modeled using GraphPad Prism™ software from GraphPad Software, Inc.



Notes

[1] Concentration of Nuclear Receptor: To reduce our use of radioactive substances and because certain radioligands are no longer commercially available, the nM concentration of active nuclear receptor is no longer determined. As of November 2012, the nM concentration reported on the Certificate of Analysis (CoA) and product label for nuclear receptor proteins is the total protein concentration as determined by a Bradford assay, and includes both active and inactive forms of the nuclear receptor. The concentration of active nuclear receptor to use in a PolarScreen™ FP assay is based on the K_d of the active receptor/Fluormone Tracer complex. The K_d and the concentration of active nuclear receptor to use in a PolarScreen™ FP assay is not lot dependent and has not changed. However, when based on total protein concentration, the recommended nM concentration of nuclear receptor to use in the PolarScreen™ FP assay will vary lot to lot. This recommended concentration corresponds to the EC_{80} (nM, total protein) determined by titration of the nuclear receptor in the presence of a constant concentration of Fluormone Tracer. The EC_{80} is reported on the CoA.

[2] Concentration of Fluormone Tracer: As of June 2013 we have updated our method for measuring the concentration of Fluormone Tracer. Originally, fluorescent intensity was used, ensuring that FP instruments would be detecting 1 nM of Fluormone Tracer with uniform intensity lot to lot. We have changed our method to measuring absorbance, which provides a much more accurate concentration of Fluormone Tracer. The physical quantity of Fluormone Tracer delivered with this kit has not changed. Rather, we have determined that the actual concentration as determined by absorbance is different than what was determined using fluorescent intensity. To be as clear and as accurate as possible, we are therefore updating the listed concentrations to the values as determined by absorbance. You will notice that the final volumes used in your assays are not affected since the actual concentration of the reagent and the recommended concentration for the assay have both been updated.

[3] The new method to calculate the concentration of Fluormone AL Green based on absorbance indicates that the concentration is 1800 nM, whereas the older method using fluorescent intensity indicated 500 nM.

[4] Optimal Concentration of Nuclear Receptor: The CoA provides the lot-specific concentration of nuclear receptor (EC_{80}) to use in the PolarScreen™ competitor assay. **We have observed that this value can be instrument dependent.** Enough nuclear receptor is included in that kit that you can check the optimal concentration for your assay. This check is optional. Refer to the CoA to determine the recommended nuclear receptor concentration. Using 0.5X, 1X, and 2X the recommended concentration of nuclear receptor, run titration curves of your control ligand and calculate the IC_{50} value for each of the curves. Prepare a table similar to the one on page 4, recording the ΔmP and the IC_{50}. Compare your results to the examples in the table and choose the optimal concentration as the 0.5X, 1X, or 2X the recommended concentration that provides the maximum (or close to maximum) mP shift without right-shifting the IC_{50} value of your control. The kit contains sufficient nuclear receptor for 1/2 the specified number of wells at 2X. In FP assays, the lower limit of IC_{50} values that can be resolved is set by the Fluormone Tracer concentration. Contact **drugdiscoverytech@lifetech.com** or call 760-603-7200, extension 40266 for further guidance.

Selection of the Optimal Concentration of Nuclear Receptor: The table below shows real examples of an FP assay and titrations of the control ligand. Each example represents a different lot of receptor. From day-to-day, with different experiments, IC_{50} values are expected to fall within ± ½ log. For the assay illustrated here, the target IC_{50} range is 9.5–95 nM. Each individual example was run on the same day and plate, so the IC_{50} range for a given example is much tighter, allowing trends in the IC_{50} to be used to optimize the assay. Concentrations of the target receptor were run at 0.5, 1.0 and 2.0X the suggested concentration for the lot. Examples 1 and 2 show cases where 2X would be recommended; an increase in ΔmP of 20–30 was obtained with little shift in the IC_{50}. Example 3 shows a case where 1X would be selected, because the IC_{50} is right-shifted with no further increase in ΔmP. Example 4 shows a case where 1X would be selected, because the increase in ΔmP is insufficient to justify the right-shift in the IC_{50} or the use of extra nuclear receptor at 2X.

Example	Concentration	(ΔmP)	IC_{50}
Example 1	0.5X	77.8	25.3
	1X	135.1	22.9
	2X	164.8	28.0
Example 2	0.5X	96.0	30.0
	1X	143.6	32.9
	2X	164.9	37.0
Example 3	0.5X	128.3	30.7
	1X	170.4	30.3
	2X	170.2	47.2
Example 4	0.5X	119.4	10.5
	1X	172.9	20.0
	2X	177.9	27.7

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

Important Licensing Information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

DISCLAIMER

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

TRADEMARKS

Life Technologies is a Thermo Fisher Scientific brand. © 2014 Thermo Fisher Scientific Inc. All rights reserved. GraphPad Prism is a trademark of GraphPad Software, Inc. Corning is a registered trademark of Corning, Inc. Cayman Chemical is a registered trademark of Cayman Chemical Company. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries.