PolarScreen™ PR Competitor Assay, Red

Catalog no. A15906 **Shipping:** Dry ice **Storage:** Varies

Publication no. MAN0009989 **Revision** A.0

Overview

PolarScreen™ Progesterone Receptor (PR) Competitor Assay, Red is a binding assay for determining the IC_{50} values of compounds that bind PR-LBD (GST). PR-LBD (GST) (Part no. A15672) is provided partially purified to retain activity. It is supplied as 0.6 mg total protein and contains sufficient active PR-LBD (GST) to run the PolarScreen™ PR Competitor Assay, Red. The A15906 kit contains enough reagents to perform the assay in up to 400 wells at 20 µL total assay volume when using the concentrations described on the lot-specific Certificate of Analysis (CoA).

Component	Composition	Storage	A15906	
			Amount	Part no.
PR-LBD (GST)[1,4]	Buffer: 50 mM Tris (pH 8.0), 1M Urea, 500 mM KCl, 1 mM EDTA, 5 mM DTT and 50% glycerol	–80°C	0.6 mg (total protein)	A15672
Fluormone PL Red[2,3] (Fluormone Tracer)	190 nM in 20 mM Tris (pH 7.6) 45% MeOH/ 55% water, **vortex prior to use**	–20°C	200 µL	P2964
Red PR Screening Buffer	Proprietary Buffer (pH 7.4), contains glycerol, **mix prior to use**	20°C to 30°C	2 × 20 mL	P2966
DTT Solution	1 M DTT in water	–20°C	1 mL	P2325

[1-4]Notes begin on page 3.

Note: Fluormone PL Red may have steroidal activity *in vivo* and therefore should be treated with caution.

Note: PR-LBD (GST) may aggregate with rough handling. Do not vortex. Minimize repeated freeze-thaw cycles, but do not create tiny aliquots. Once thawed, PR-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 low-volume 384-well plates with NBS surface (Corning®, Cat. no. 4514).
- We recommend a PR ligand, such as Progesterone or R5020 (PerkinElmer®, Cat. no. NPD-004005) as the control ligand.
- The K_d of the Fluormone PL Red with PR-LBD (GST) is approximately 19 nM (based on **active** receptor).
- Fluromone PL Green is hydrophobic. Use polypropylene pipette tips for all manipulations. Avoid using polystyrene pipettes.
- PR-LBD (GST)/Fluormone PL Green complexes should be prepared in glass or high-density polyethylene (HDPE) when glass is not available because of the hydrophobic nature of Fluormone PL Red. The complex should be stored on ice until dispensed.
- Complete PR Screening Buffer Green with DTT must be used the day it is prepared. For every 1 mL of thawed Red PR Screening Buffer add 2 µL of 1 M DTT. Vortex.

FAST FACTS, continued

- Solvent tolerance, 1% DMSO final preferred, up to 1.25% DMSO, 1.25% MeOH, 0.5% EtOH may be used.
- Incubate assays at room temperature for 2–4 hours. Plate can be read during a 4-hour window. Use consistent time.

Final assay conditions

Reagent	1X Final assay concentration
PR-LBD (GST)	See lot specific CoA[4]
Fluormone PL Red	3.8 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 PR LBD (GST)/Fluormone Complex.

Reagent volumes

The table below summarizes the reagent amounts required for performing the PolarScreen™ PR-LBD (GST) Competitor Assay, Red 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 control ligand (20 µM)	—	—	—	10 µL
2X Test Compound (single points or titrations)	10 µL	—	—	—
2X PR-LBD (GST) /Fluormone PR Red Complex	10 µL	—	10 µL	10 µL
2X Fluormone PL Red	—	10 µL	—	—
PR Screening Buffer, Green Assay 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

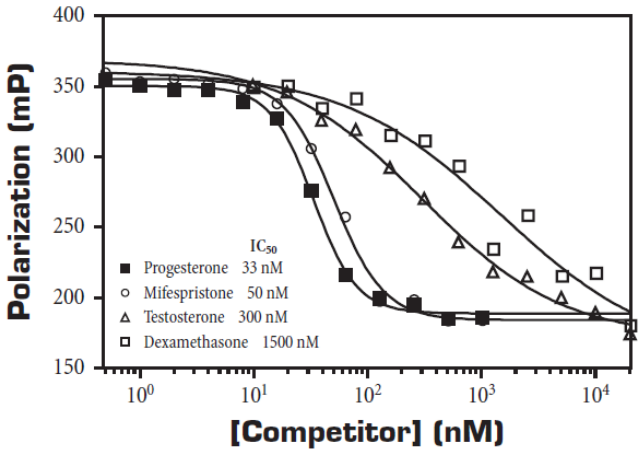
Note: Refer to the **PolarScreen™ Nuclear Receptor Competitor Assays - Universal Protocol** at **www.lifetechnologies.com** for assay plate layout and for detailed instructions on preparing and delivering the reagents.

1. Add the reagents listed in the table above into the appropriate wells of the assay plate.
2. Mix the assay plate.
3. Cover plate to protect reagents from light.
4. Incubate plate at room temperature for 2 hours.
5. Measure fluorescence polarization value (mP) of each well on a fluorescence polarization plate reader within 1– 4 hours of mixing the reagents. Use consistent time. After 4 hours polarization values will start to decrease.

Example data

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

PolarScreen™ PR Competitor Assay, Red



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 PL Red based on absorbance indicates that the concentration is 190 nM, whereas the older method using fluorescent intensity indicated 100 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

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