



## PolarScreen™ GR Competitor Assay, Red

Catalog no. A15898

Shipping: Dry ice

Storage: Varies

Publication no. MAN0008942

Revision B.0

### Overview

PolarScreen™ Glucocorticoid Receptor (GR) Competitor Assay, Red is a binding assay for determining the IC<sub>50</sub> values of compounds that bind the full length GR. GR Full Length (Part no. A15663) is provided partially purified to retain activity. It is supplied as 8.3 mg total protein and contains sufficient active GR Full Length to run the PolarScreen™ GR Competitor Assay, Red. The A15898 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	A15898	
			Amount	Part no.
GR Full Length <sup>1</sup>	Buffer: 10 mM potassium phosphate (pH 7.4), 5 mM DTT, 0.1 mM EDTA, 10 mM Na <sub>2</sub> MoO <sub>4</sub> , and 10% glycerol Storage buffer is yellow in color due to the formation of DTT/Molybdate complex.	-80°C	8.3 mg (total protein)	A15663
Fluormone GS Red <sup>2,3</sup> (Fluormone Tracer)	270 nM in 55% MeOH, <b>vortex prior to use</b>	-20°C	50 µL	P2894
GR Stabilizing Peptide, 10X	1 mM in 10 mM potassium phosphate (pH 7.4), <b>vortex prior to use</b>	-80°C	3 × 1 mL	P2815
GR Screening Buffer, 10X	100 mM potassium phosphate (pH 7.4), 200 mM Na <sub>2</sub> MoO <sub>4</sub> , 1 mM EDTA and 20% DMSO, <b>mix prior to use</b>	20°C to 30°C	3 × 1 mL	P2814
DTT Solution	1 M DTT in water	-20°C	1 mL	P2325

<sup>1-4</sup>Notes begin on page 3.

**Note:** GR Full Length may aggregate with rough handling. Do not vortex. Do not expose GR Full Length to more than 8 freeze-thaw cycles. Once thawed, GR Full Length must remain on ice.

### FAST FACTS

- For more detailed instruction on running a PolarScreen™ Nuclear Receptor Competitor Assay, go to [www.lifetechnologies.com](http://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](http://www.lifetechnologies.com/nuclearreceptor).
- We recommend using low-volume 384-well plates with NBS surface (Corning®, Cat. no. 4514).
- Do not freeze GR Full Length on dry ice as the product is sensitive to pH shifts.
- We recommend a GR ligand such as Dexamethasone (Sigma-Aldrich®, Cat. no. D4902), IC<sub>50</sub> 10 nM ± ½ log as the control ligand.
- Stabilizing Peptide is necessary for running the PolarScreen™ GR Competitor Assay, Red at room temperature. Diluted GR Full Length is unstable at temperatures > 8°C.
- The K<sub>d</sub> of the Fluormone GS Red with full length GR equals 0.4 nM ± 0.1 nM (based on active receptor). For adequate detection 1.4 nM of Fluormone GS Red is required.

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## FAST FACTS, continued

- Do not premix GR Full Length and Fluormone GS Red. Once the Fluormone GS Red/GR Complex has been formed, competition by test compounds is relatively slow. Add GR to wells after mixing Fluormone GS Red and test compound.
- Complete GR Screening Buffer with DTT and GR Stabilizing Peptide must be used the day it is prepared. Mix 1 mL of thawed GR Screening Buffer 10X, 1 mL of GR Stabilizing Peptide 10X, 50 µL to 7.95 mL water and vortex.
- Solvent tolerance, 1% DMSO final preferred, up to 5% DMSO and 5% MeOH may be used.
- Incubate assays at room temperature for two hours and read within 2–4 hours of mixing the reagents. Use consistent time.

## Final assay conditions

Reagent	1X Final assay concentration
GR Full Length	See lot specific CoA <sup>4</sup>
Fluormone GS Red	1.4 nM

<sup>4</sup> We have observed that the optimal concentration of the nuclear receptor can be instrument dependent. See Note 4, page 4, for additional details.

## Quick start protocol

**Note:** Do not premix GR Full Length and the Fluormone Tracer.

**Note:** Do not vortex the GR Full Length.

## Reagent volumes

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

Component	Assay	Controls		
	Test Compound	No Receptor Control (Free Fluormone)	Maximum mP Control	Minimum mP Control (Displaced Fluormone Tracer)
2X Saturating Dexamethasone (20 µM)	—	—	—	10 µL
2X Test Compound (single points or titrations)	10 µL	—	—	—
4X Fluormone GS Red	5 µL	—	5 µL	5 µL
4X GR Full Length	5 µL	—	5 µL	5 µL
2X Fluormone GS Red	—	10 µL	—	—
Complete GR 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 ( $\Delta$ mP), is the difference between the Maximum mP Control and Minimum mP Control of displaced Fluormone Tracer; see table above.

## Perform the assay

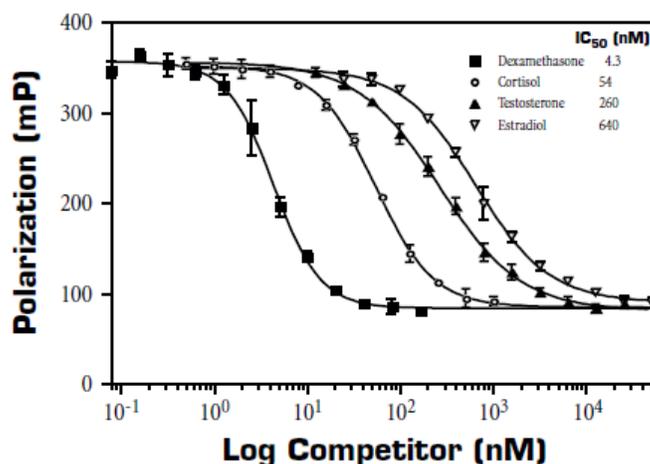
**Note:** Refer to the PolarScreen™ Nuclear Receptor Competitor Assays - Universal Protocol at [www.lifetechnologies.com](http://www.lifetechnologies.com) for assay plate layout and for detailed instructions on preparing and delivering the reagents.

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 at least 2 hours.
5. Measure fluorescence polarization value (mP) of each well on a fluorescence polarization plate reader within 2 – 4 hours of mixing the reagents.

## Example data

An example of competitive binding data generated using the PolarScreen™ GR 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™ GR Competitor Assay, Red



## Notes

**[1] Optimal Concentration of Nuclear Receptor:** The CoA provides the lot-specific concentration of nuclear receptor (EC<sub>80</sub>) 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<sub>50</sub> value for each of the curves. Prepare a table similar to the one on page 4, recording the ΔmP and the IC<sub>50</sub>. 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<sub>50</sub> value of your control. The kit contains sufficient nuclear receptor for ½ the specified number of wells at 2X. In FP assays, the lower limit of IC<sub>50</sub> values that can be resolved is set by the Fluormone Tracer concentration. Contact [drugdiscoverytech@lifetech.com](mailto:drugdiscoverytech@lifetech.com) or call 760-603-7200, extension 40266 for further guidance.

### [2] Note on the 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 absorbance, as this gives a much more accurate determination of the concentration of

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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 GS Red based on absorbance indicates that the concentration is 270 nM, whereas the older method using fluorescent intensity indicated 200 nM.

**[4] 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<sub>50</sub> values are expected to fall within  $\pm \frac{1}{2}$  log. For the assay illustrated here, the target IC<sub>50</sub> range is 9.5–95 nM. Each individual example was run on the same day and plate, so the IC<sub>50</sub> range for a given example is much tighter, allowing trends in the IC<sub>50</sub> 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  $\Delta mP$  of 20–30 was obtained with little shift in the IC<sub>50</sub>. Example 3 shows a case where 1X would be selected, because the IC<sub>50</sub> is right-shifted with no further increase in  $\Delta mP$ . Example 4 shows a case where 1X would be selected, because the increase in  $\Delta mP$  is insufficient to justify the right-shift in the IC<sub>50</sub> or the use of extra nuclear receptor at 2X.

Example	Concentration	( $\Delta mP$ )	IC <sub>50</sub>
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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