PolarScreen™ GR Competitor Assay, Green

Catalog no. A15897 **Shipping:** Dry ice **Storage:** Varies
Publication no. MAN0009992 **Revision** B.0

Overview

PolarScreen™ Glucocorticoid Receptor (GR) Competitor Assay, Green is a binding assay for determining the IC_{50} 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 full length GR to run the PolarScreen™ GR Competitor Assay, Green. The A15897 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	A15897	
			Amount	Part no.
GR Full Length[1]	Buffer: 10 mM potassium phosphate (pH 7.4), 5 mM DTT, 0.1 mM EDTA, 10 mM Na_2MoO_4 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 GS1[2,3] (Fluormone Tracer)	500 nM in 20 mM Tris, 90% methanol, **vortex prior to use**	–20°C	50 µL	P2813
GR Stabilizing Peptide, 10X	1 mM in 10 mM potassium phosphate (pH 7.4), **vortex prior to use**	–80°C	3 × 1 mL	P2815
GR Screening Buffer, 10X	100 mM potassium phosphate (pH 7.4), 200 mM Na_2MoO_4, 1 mM EDTA and 20% DMSO, **mix prior to use**	20°C to 30°C	3 × 1 mL	P2814
DTT Solution	1 M DTT in water	–20°C	1 mL	P2325

[1-4]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**, 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).
- 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_{50} 10 nM ± ½ log as the control ligand
- Stabilizing Peptide is necessary for running the PolarScreen™ GR Competitor Assay, Green at room temperature. Diluted GR Full Length is unstable at temperatures > 8°C.
- The K_d of the Fluormone GS1 with full length GR equals 0.75 nM ± 0.25 nM (based on **active** receptor). For adequate detection 2.5 nM of Fluormone GS1 is required.

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

Thermo Fisher Scientific Inc • 5791 Van Allen Way • Carlsbad, CA 92008 • Phone: 760 603 7200 • FAX: 760 602 6500
www.lifetechnologies.com

FAST FACTS, continued

- Do not premix full length GR and Fluormone GS1. Once the Fluormone GS1/GR Complex has been formed, competition by test compounds is relatively slow. Add GR to wells after mixing Fluormone GS1 and test compounds.
- 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 1 M DTT 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[4]
Fluormone GS1	2.5 nM

[4] 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, 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 Dexamethasone (20 µM)	—	—	—	10 µL
2X Test Compound (single points or titrations)	10 µL	—	—	—
4X Fluormone GS1	5 µL	—	5 µL	5 µL
4X GR Full Length	5 µL	—	5 µL	5 µL
2X Fluormone GS1	—	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 (ΔmP), is the difference between the ***Maximum mP Control*** and ***Minimum mP Control*** of displaced Fluormone Tracer; see table above.

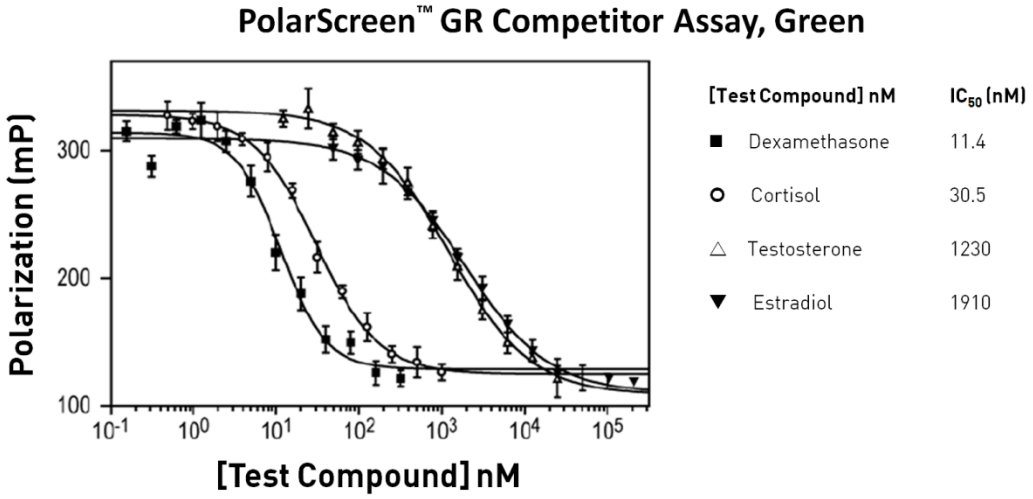
Perform the assay

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 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, 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] 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 ½ 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.

[2] Note on the Concentration of Fluormone Tracer: As of November 2009, 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 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 GS1 based on absorbance indicates that the concentration is 500 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_{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

For Technical Support for this or other Drug Discovery Products, dial 760-603-7200, extension 40266.