



PolarScreen™ ER Beta Competitor Assay, Green

Catalog nos. A15889, A15890

Shipping: Dry Ice

Storage: Varies

Publication no. MAN0008943

Revision A.0

Overview

PolarScreen™ Estrogen Receptor Beta (ER Beta) Competitor Assay, Green is a binding assay for determining the IC₅₀ values of compounds that bind the full-length ER Beta. When using the concentrations described on the lot-specific Certificate of Analysis (CoA), the:

- A15889 kit contains enough reagents to perform the assay in up to 2500 wells at 20 µL total assay volume
- A15890 kit contains enough reagents to perform the assay in up to 800 wells at 20 µL total assay volume

Component	Composition	Storage	A15890		A15889	
			Amount	Part no.	Amount	Part no.
ER Beta Full Length ¹	Buffer: 50 mM Bis-Tris-Propane (pH 9.0), 500 mM KCl, 50% Glycerol, 275 mM Urea, 0.6% (w/v) CHAPS, 2 mM DTT	-80°C	180 µg	A15664	2 × 180 µg	A15664
Fluormone™ ES2 Green ² (Fluormone™ Tracer)	1800 nM ³ in 80% methanol/20% water	-20°C	40 µL	PV6045	125 µL	P2645
ES2 Screening Buffer	Proprietary Buffer (pH 7.4), 0% Glycerol	20–30°C	120 mL	P2616	120 mL	P2616

¹⁻³ See notes 1–3 on changes in concentration determinations for the nuclear receptor and Fluormone™ Tracer, beginning on page 3.

Note: Fluormone™ ES2 Green may have estrogenic activity *in vivo* and therefore should be handled with caution.

Note: ER Beta protein may aggregate with rough handling. Do not vortex. Do not expose ER Beta to more than 3 freeze-thaw cycles. Once thawed, ER Beta 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 part number, and view manuals.
- For information on Life Technologies' Nuclear Receptor Portfolio, visit www.lifetechnologies.com/nuclearreceptor.
- We recommend using low-volume 384-well, black, round-bottom polystyrene plates, not treated (Corning®, Cat. no. 4511).
- We recommend an ER Beta ligand, such as Estradiol (Sigma, Cat. no. E1024), as the control ligand.
- The K_d of the Fluormone™ ES2 Green with ER Beta Full Length equals 18 ± 9 nM of active receptor.
- Incubate assays at room temperature for 2 hours, after which the plate can be read during a 7-hour window. Use consistent time.

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Final Assay Conditions

Reagent	1X Final assay concentration
ER Beta Full Length	See lot-specific CoA ⁴
Fluormone™ ES2 Green (Fluormone™ Tracer)	4.5 nM

⁴ We have observed that the optimal concentration of the nuclear receptor can be instrument-dependent. See Note 4 on page 3 for additional details.

Quick Start Protocol

Reagent volumes

The table below summarizes the reagent amounts required for performing the PolarScreen™ ER Beta Competitor Assay, Green and the associated controls at 20 μ L 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 Estradiol (20 μ M)	—	—	—	10 μ L
2X Test Compound (single points or titrations)	10 μ L	—	—	—
2X ER Beta/Fluormone™ ES2 Green Complex	10 μ L	—	10 μ L	10 μ L
2X Fluormone™ ES2 Green	—	10 μ L	—	—
Complete ES2 Screening Buffer with 2X DMSO (or other solvent)*	—	10 μ L	10 μ L	—

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

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

Performing the assay

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

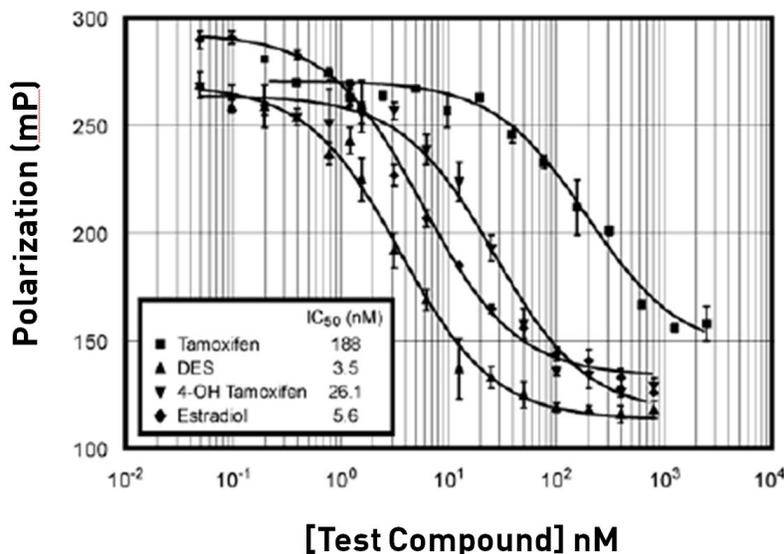
1. Add the reagents listed in the table above into the appropriate wells of the assay plate.
2. Mix the assay plate.
3. Cover the plate to protect the reagents from light.
4. Incubate the plate at room temperature for at least 2 hours.
5. Measure the fluorescence polarization value (mP) of each well on a fluorescence polarization plate reader within 7 hours of mixing the reagents.

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Example Data

An example of competitive binding data generated using the PolarScreen™ ER Beta 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.

PolarScreen™ ER Beta Competitor Assay, Green



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 EC₈₀ (nM, total protein) determined by titration of the nuclear receptor in the presence of a constant concentration of Fluormone™ Tracer. The EC₈₀ is reported on the CoA.

[2] **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 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 from 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 determined by absorbance. You will notice that the final volumes used in your assays are not affected because 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™ ES2 Green based on absorbance indicates that the concentration is 1800 nM, whereas the older method using fluorescent intensity indicated 400 nM.

[4] **Optimal Concentration of Nuclear Receptor:** The CoA provides the lot-specific concentration of nuclear receptor (EC₈₀) to use in the PolarScreen™ competitor assay. **We have observed that this value can be instrument-dependent.** Enough nuclear receptor is included in the 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 IC₅₀ value for each of the curves. Prepare a table

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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 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 $\frac{1}{2}$ 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.

[5] 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 $\pm \frac{1}{2}$ 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 0.5X, 1.0X, 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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