PolarScreen™ ER Alpha Competitor Assay, Red

Catalog no. A15884 **Shipping:** Dry Ice **Storage:** Varies

Publication no. MAN0009428 **Revision** A.0

Overview

PolarScreen™ Estrogen Receptor Alpha (ER Alpha) Competitor Assay, Red is a binding assay for determining the IC_{50} values of compounds that bind the full-length ER Alpha. When using the concentrations described on the lot-specific Certificate of Analysis (CoA), the A15884 kit contains enough reagents to perform the assay in up to 800 wells at 20 µL total assay volume.

Component	Composition	Storage	A15884	
			Amount	Part no.
ER Alpha Full Length[1]	Buffer: 50 mM Tris-HCl (pH 8.0), 500 mM KCl, 2 mM DTT, 1 mM EDTA, 1 mM Na_3VO_4, 10% Glycerol	–80°C	140 µg	A15674
Fluormone™ EL Red[2] (Fluormone™ Tracer)	285 nM in 20 mM Tris, 90% Methanol	–20°C	100 µL	P3030
ER Red Screening Buffer	Proprietary Buffer (pH 8.0), 10% Glycerol	20–30°C	2 × 25mL	P3031

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

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

Note: ER Alpha Full Length may aggregate with rough handling. Do not vortex. Do not expose ER Alpha to more than 3 freeze-thaw cycles. Once thawed, ER Alpha 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 Alpha ligand, such as Estradiol (Sigma, Cat. no. E1024), as the control ligand.
- The K_d of the Fluormone™ EL Red with ER Alpha Full Length equals 7–14 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.

Final Assay Conditions

Reagent	1X Final assay concentration
ER Alpha Full Length	See lot-specific CoA[4]
Fluormone™ EL Red (Fluormone™ Tracer)	1.4 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 Alpha Competitor Assay, Red and the associated controls at 20 µL total assay volume.

	Assay	Controls		
Component	Test compound	No Receptor Control (free Fluormone™ Tracer Control)	Maximum mP Control	Minimum mP Control (displaced Fluormone™ Tracer)
2X Saturating Estradiol (20 µM)	—	—	—	10 µL
2X Test Compound (single points or titrations)	10 µL	—	—	—
2X ER Alpha/Fluormone™ EL Red Complex	10 µL	—	10 µL	10 µL
2X Fluormone™ EL Red	—	10 µL	—	—
Complete ER Red 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 the table above.

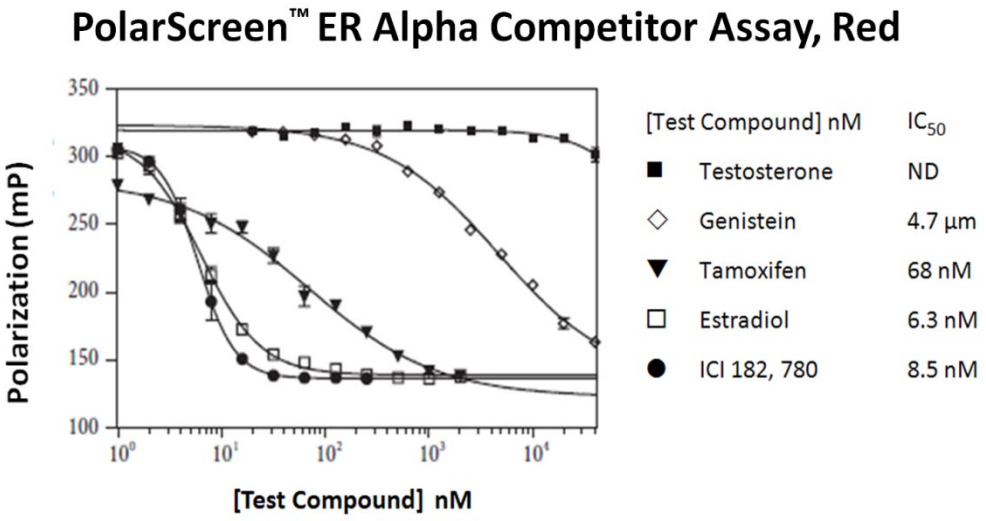
Performing the assay

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.

Example Data

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



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 July 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 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™ EL Red based on absorbance indicates that the concentration is 285 nM, whereas the older method using fluorescent intensity indicated 200 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 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 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 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 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 ± 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 0.5X, 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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