

Thermo Scientific ER β Redistribution[®] Assay

The Redistribution technology monitors the cellular translocation of GFP-tagged proteins in response to drug compounds or other stimuli and allows easy acquisition of multiple readouts from the same cell in a single assay run. In addition to the primary readout, high content assays provide supplementary information about cell morphology, compound fluorescence, and cellular toxicity.

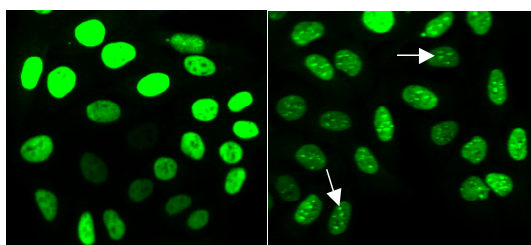


Figure 1. Nuclear foci formation of EGFP-ER β . Cells were treated with 100 nM 17 β -estradiol for 20 hrs (right panel) or untreated (DMSO control, left panel). Arrows indicate the nuclear foci containing EGFP-ER β that are detected by the image analysis algorithm.

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Estrogen is a regulator of normal endocrine functions. Signal transduction induced by estrogens such as estradiol, the main endogenous human estrogen, is mediated by the estrogen receptor (ER). ER is a nuclear receptor that upon ligand binding organizes into homo- and heterodimers of the ER α and ER β subtypes. Following ligand binding, ER acts as a transcription factor and regulates expression of several target genes such as cyclin D1 and IGF-1 [1]. Estrogens and estrogen receptors are implicated in development and progression of breast cancer. Moreover, environmental chemical contaminants with estrogenic activity are suggested to promote reproductive disorders. The EC₅₀ of 17 β -estradiol in the assay is approximately 0.6 nM and corresponds well with EC₅₀ values reported by others [2].

Features

- Designed to assay compounds for their ability to modulate accumulation of ER β in nuclear foci
- Coupled to EGFP for easy monitoring of the cellular translocation event
- Robust cell-based assay for use in high content analysis and fluorescence microscope applications

Highlights:

- **Biologically relevant data**
Compounds tested in a cellular environment
- **Validated**
Functionally tested cells provided with an optimized assay protocol
- **Easy to use**
Just plate cells, add compounds, and image

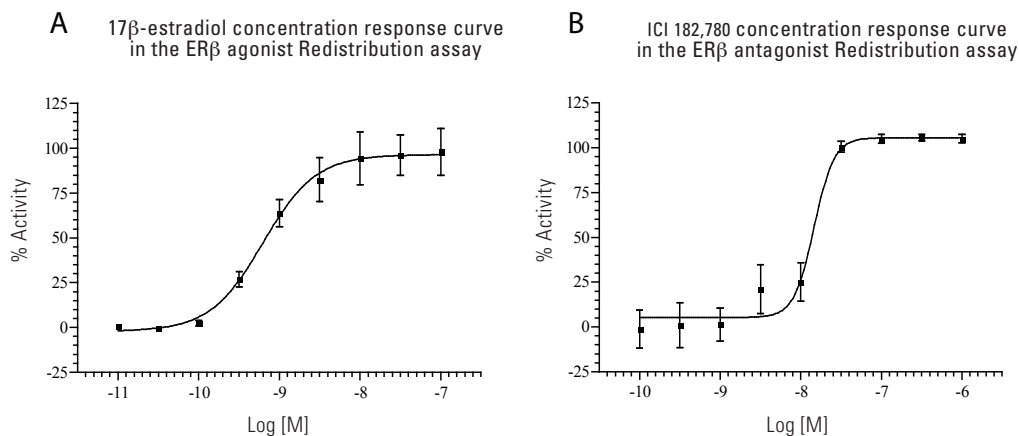


Figure 2. Concentration response curves in the ER β assay: A) 17 β -estradiol concentration response in the ER β agonist assay (n = 16). The EC₅₀ is approximately 0.6 nM. Concentration response was measured in 9 point half log dilution series. Cells were treated with 17 β -estradiol for 20 hr. Cells were then fixed and nuclear foci formation was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (100 nM 17 β -estradiol) and negative control (0.25% DMSO). **B)** ICI 182,780 concentration response in the ER β antagonist assay (n = 16). The EC₅₀ is approximately 15 nM. Cells were treated with 17 β -estradiol in the presence of a half log dilution series of ICI 182,780 for 20 hr. Cells were then fixed and nuclear foci formation was measured using the IN Cell Analyzer 3000 (GE Healthcare). % activity was calculated relative to the positive (1 μ M ICI 182,780) and negative control (0.25% DMSO)

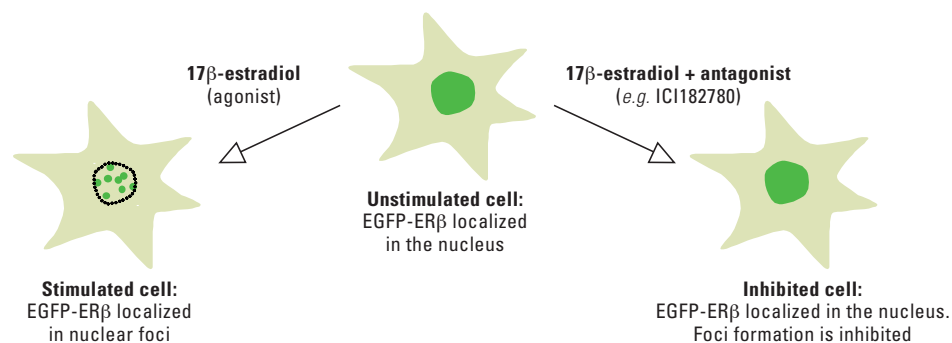


Figure 3. Illustration of the ER β translocation event.

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Assay Details

Recombinant U2OS cells stably expressing human estrogen receptor ER β fused to the C-terminus of enhanced green fluorescent protein (EGFP). The assay monitors accumulation of an EGFP-ER β fusion protein in nuclear foci as a measure of ER β activation. The agonist version of the assay uses the potent estrogen 17 β -estradiol as a reference compound. The antagonist version of the assay uses the estrogen receptor antagonist ICI182,780 as a reference compound. The ER β assay is validated with an average $Z' = 0.55 \pm 0.12$ (agonist format), $Z' = 0.38 \pm 0.06$ (antagonist format), suitable for both profiling and screening applications.

Imaging

The translocation of EGFP-ER β can be imaged on most HCS platforms and fluorescence microscopes. The filters should be set for Hoechst (350/461 nm) and GFP/FITC (488/509 nm) (wavelength for excitation and emission maxima). Consult the instrument manual for

the correct filter settings. The translocation can typically be analyzed on images taken with a 20x objective or higher magnification. The primary output in the ER β Redistribution assay is the formation of spots in the nucleus. The data analysis should therefore report an output that corresponds to number, area, or intensity of spots in the nucleus.

Imaging on Thermo Scientific Cellomics ArrayScan V^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} using a 20x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the SpotDetectorV3 BioApplication. The output parameter used was SpotTotalAreaPerObject. The minimally acceptable number of cells used for image analysis in each well was set to 150 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

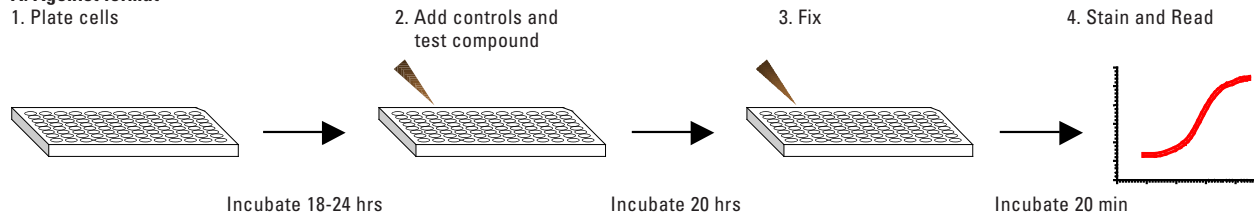
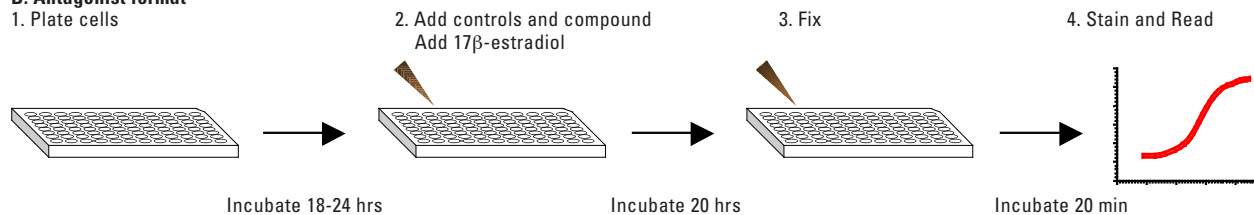
A. Agonist format**B. Antagonist format**

Figure 4. The ER β Redistribution assay is very easy and fast to perform in both agonist and antagonist format.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
063_01	ER β Redistribution Assay	U2OS	•	•	•

The Redistribution Assays are available in 3 product formats, Profiling, Screening and CryoRedi, for different volume and level of convenience needs. The Redistribution Assays can also be accessed through the Thermo Scientific Managed Services.

Related Thermo Scientific Products

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
043_01	AR Redistribution Assay	U2OS	•	•	•
060_01	VDR Redistribution Assay	U2OS	•	•	•
056_01	ER α Redistribution Assay	U2OS	•	•	•
014_01	GR Redistribution Assay	U2OS	•	•	•
064_01	PR Redistribution Assay	U2OS	•	•	•
077_01	PXR Redistribution Assay	U2OS	•	•	•
062_01	REV Redistribution Assay	U2OS	•	•	
CX03004-INS	Cellomics ONE BioApplication Suite	High content data acquisition and analysis software			
CX03102A/B	Cellomics ArrayScan V ^{II}	Flexible, high throughput, high content reader			
N01-3001	CellWoRx	Economical high content reader			

References

1. Ellis R Levin. *Molecular Endocrinology*. 19(8):1951-1959, 2005.
2. Wilson S V. et al. *Toxicological Sciences*. (81):69-77, 2004.

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