

Thermo Scientific FSHR 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.

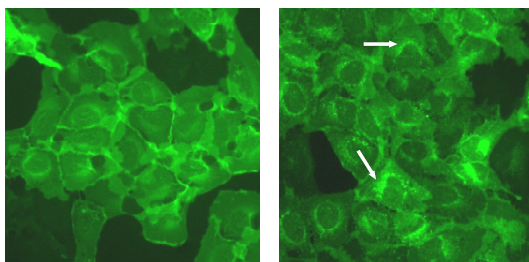


Figure 1. Internalization of rFSHR-EGFP stimulated with FSH. Cells were untreated (DMSO control, left panel) or treated with 20 U/ml recombinant human FSH for 2 hr (right panel). Arrows indicate the rFSHR-EGFP internalization that is detected by the image analysis algorithm.

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The development of ovulatory follicles involves differentiation of the granulosa cell component from a mitotically active, estrogen-secreting type (immature cells) into a non-dividing, luteinized, progesterone-secreting type (mature cells). Granulosa cell differentiation is regulated by two pituitary hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH), as well as intra-ovarian steroids and growth factors.

The follitropin, lutropin, and thyrotropin receptors (FSHR, LHR, and TSHR) belong to the family of GPCRs. They form a small sub-family of GPCRs, collectively known as the leucine-rich repeat containing GPCRs, that is characterized by the presence of relatively large extracellular domains composed of leucine-rich repeats. FSH-induced activation of the FSHR results in the internalization of the FSH-FSHR complex via clathrin-coated pits by a pathway that requires dynamin [1, 2]. As FSHR couples to $G\alpha_s$, FSH stimulation of FSHR leads to activation adenylate cyclase which in turn catalyzes the formation of cAMP from ATP.

Features

- Designed to assay compounds for their ability to modulate internalization of FSHR
- 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

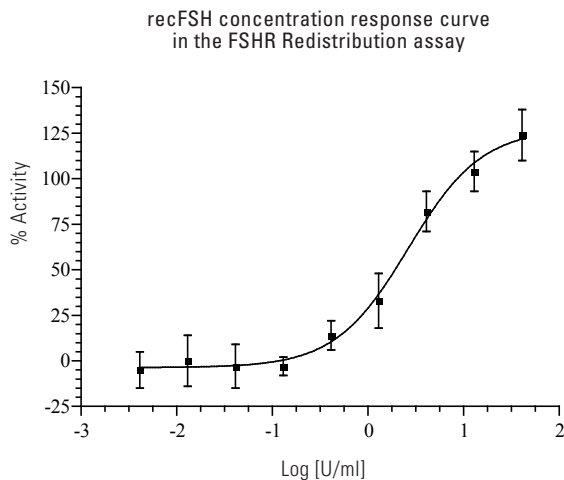


Figure 2. FSH concentration response in the FSHR Redistribution assay. Concentration response was measured in 9 point half log dilution series ($n = 8$). Cells were incubated with recFSH for 2 hr. Cells were then fixed and receptor internalization was measured using the Cellomics ArrayScan V^{TI} Reader and the SpotDetectorV3 BioApplication. % activity was calculated relative to the positive (20 U/ml recFSH) and negative control (0.25% DMSO). The EC_{50} of recFSH is ~ 2.5 U/ml.

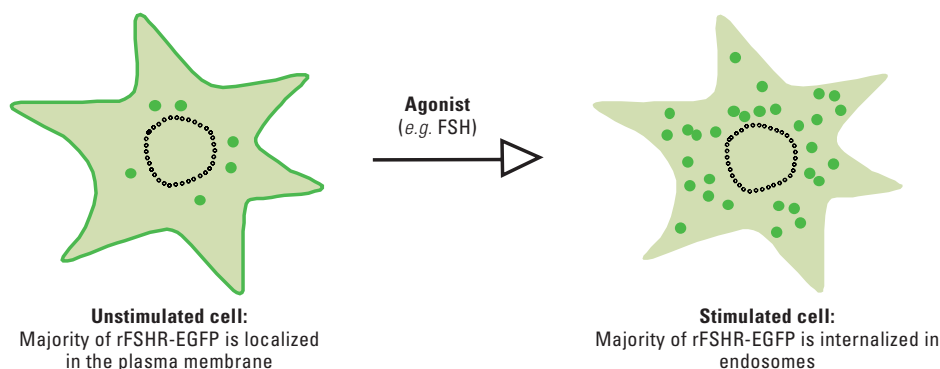


Figure 3. Illustration of the FSHR β translocation event.

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Assay Details

Recombinant U2OS cells stably expressing rat Follicle Stimulating Hormone Receptor (rFSHR) fused to the N-terminus of enhanced green fluorescent protein (EGFP). The FSHR internalization assay is based on rat FSHR (rFSHR) and is designed to screen for agonists of FSHR translocation by monitoring the internalization of a membrane-localized rFSHR-GFP fusion protein to endosomes. Test compounds are assayed for their ability to induce FSHR internalization by a spot detecting image analysis algorithm. The FSHR assay is validated with an average $Z' = 0.57 \pm 0.06$, suitable for both screening and profiling applications.

Imaging

The translocation of rFSHR-GFP 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 FSHR Redistribution assay is the formation of spots in the cytoplasm. The data analysis should therefore report an output that corresponds to number, area or intensity of spots in the cytoplasm.

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 200 cells. Other BioApplications that can be used for this assay include CompartmentalAnalysisV2 and ColocalizationV3.

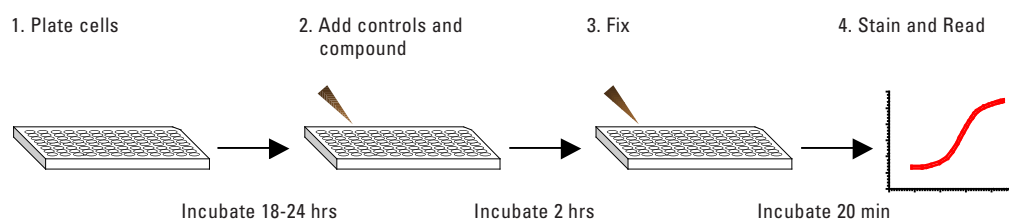


Figure 4. The FSHR Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
053_01	FSHR 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
067_01	CXCR4 Redistribution Assay	U2OS	•	•	
094_01	GRPR Redistribution Assay	U2OS	•	•	
054_01	MCH1R Redistribution Assay	U2OS	•		
039_01	S1P1 Redistribution Assay	U2OS	•	•	•
095_01	S1P3 Redistribution Assay	U2OS	•		•
086_01	M1 Redistribution Assay	U2OS	•		
075_01	M2 Redistribution Assay	U2OS	•	•	
076_01	M3 Redistribution Assay	U2OS	•	•	
057_01	MC4 Redistribution Assay	U2OS	•		
093_01	CRTH2 Redistribution Assay	U2OS	•	•	
051_01	CB1 Redistribution Assay	U2OS	•	•	•
061_01	CB2 Redistribution Assay	U2OS	•		•
097_02	GLP1R Redistribution Assay	U2OS	•	•	
8404301	Cellomics PKA Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8401501	Cellomics Phospho-CREB HCS Reagent Kit	Antibody- and dye-based reagent kit			
K0100071	Cellomics ERK MAPK Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
CX03004-INS	Cellomics ONE BioApplication Suite	High content data acquisition and analysis software			
CX03102A/B	Cellomics ArrayScan V ^{TI}	Flexible, high throughput, high content reader			
N01-3001	CelliWoRx	Economical high content reader			

References

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2. Krejci A et al. *Physiol Res.*; 53, S131-40, 2004.
3. Eglen RM *Auton Autacoid Pharmacol.*; 26, 219-33, 2006.

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