

Thermo Scientific Rev 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.

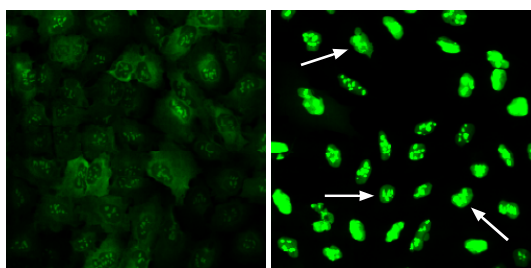


Figure 1. Nuclear translocation of Rev-EGFP. Cells were treated with 30 nM Ratjadone A for 1 hr (right panel) or untreated (DMSO control, left panel). Arrows indicate nuclear/nucleolar accumulation of Rev-EGFP detected by the image analysis algorithm.

Thermo Scientific Rev Redistribution Assay

The human immunodeficiency virus type 1 (HIV-1) regulatory protein, Rev, is an RNA-binding protein essential for the expression of viral structural proteins and productive infection. Rev contains a nuclear export signal (NES) in its C-terminal domain and a nuclear localization signal (NLS) in its N-terminal domain. The NES and NLS are necessary for shuttling between nucleus and cytoplasm and are therefore crucial for the transport of unspliced and singly spliced viral transcripts. Nuclear export of Rev protein is dependent on the classical NES/Crm1 pathway that regulates the continuous shuttling of many proteins between the nucleus and the cytoplasm [1].

In the Rev Redistribution assay the Rev sequence is fused to the NES of the human protein kinase inhibitor alpha in order to induce a clear cytoplasmic localization of Rev in untreated cells. Nuclear accumulation of Rev can be obtained by specific inhibitors of Crm1-mediated nuclear export such as Ratjadone A [2].

Features

- Designed to assay compounds for their ability to inhibit nuclear export of Rev
- 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

Concentration response curves in the Rev Redistribution assay

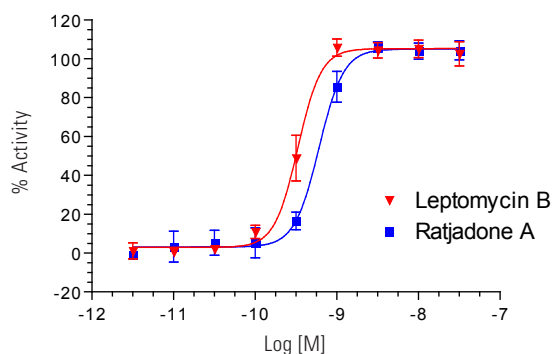


Figure 2. Concentration response curves in the Rev assay. Concentration response was measured in 9 point half log dilution series (n = 8). The EC₅₀ of Leptomycin B is ~0.3 nM, and the EC₅₀ of Ratjadone A is ~0.6 nM. Cells were incubated with compound for 1 hr. Cells were then fixed and membrane translocation was measured using the Cellomics ArrayScan V[™] Reader and the Redistribution V3 BioApplication. % activity was calculated relative to the positive (30 nM Ratjadone A) and negative control (0.25% DMSO).

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

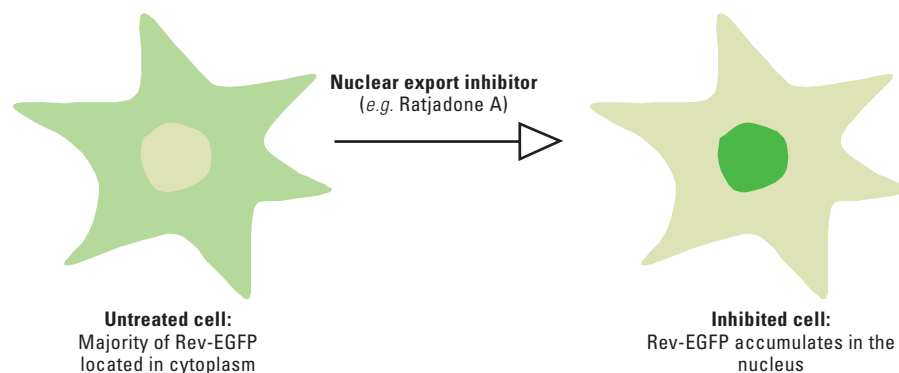


Figure 3. Illustration of the Rev translocation event.

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Assay Details

Recombinant U2OS cells stably expressing Rev fused to the N-terminus of enhanced green fluorescent protein (EGFP). The assay is designed to test for general inhibitors of Crm1-dependent export by monitoring the translocation of the Rev-EGFP fusion protein from the cytoplasm to a nuclear/nucleolus localization. Ratjadone A is used as reference compound. The activity of Ratjadone A in the assay is approximately equivalent to the activity obtained with the natural export inhibitor Leptomycin B. The assay can also be used as a selectivity assay for nuclear translocation assays such as FKHR or MK2 to deselect compounds that are general export inhibitors. The Rev assay is validated with an average $Z' = 0.70 \pm 0.11$, suitable for both screening and profiling applications.

Imaging

The translocation of Rev-EGFP 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 10x objective or higher magnification. The

primary output in the Rev Redistribution assay is the translocation of Rev-EGFP from the cytoplasm to the nucleus. The data analysis should therefore report an output relating to the GFP fluorescence intensities in the nucleus and the cytoplasm.

Imaging on Thermo Scientific Cellomics ArrayScan V[®]

This assay has been validated on the Cellomics ArrayScan V[®] using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the Redistribution V3 BioApplication. The output used was MEAN_CircRingAvgIntenRatioLog (Log of the ratio of average fluorescence intensities of nucleus and cytoplasm (well average)). 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 Molecular TranslocationV2, CompartmentalAnalysisV2, NucTransV2, and ColocalizationV3.

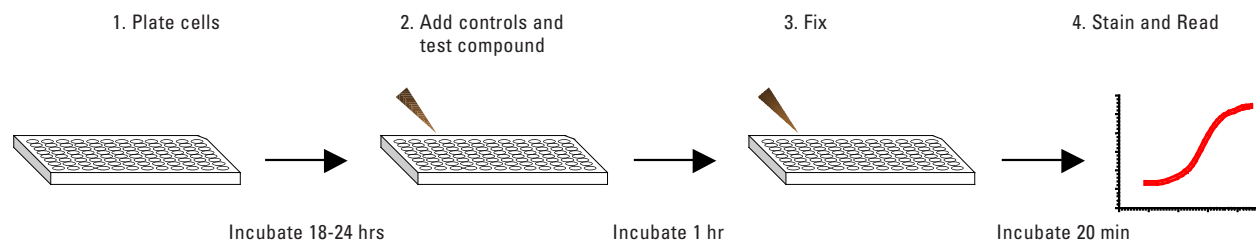


Figure 4. The Rev Redistribution assay is very easy and fast to perform.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
062_01	Rev 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
008_01	FKHR (FOXO1) Redistribution Assay	U2OS	•	•	•
009_02	FKHRL1 (FOXO3a) Redistribution Assay	U2OS	•	•	•
090_01	AFX/Foxo4 Redistribution Assay	U2OS	•	•	
037_01	MK2 Redistribution Assay	U2OS	•	•	•
8407201	Cellomics FOXO1A Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8401701	Cellomics FOXO3a activation 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	CellWoRx	Economical high content reader			

References

1. Neville M. et al. *Curr. Biol.* 7, 767-775, 1997.
2. Kalesse M. et al. *Chembiochem.* 2, 709-714, 2001.

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