Product Specifications

# Thermo Scientific Rev nuclear export Redistribution® Assay (BHK-21)

**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.**

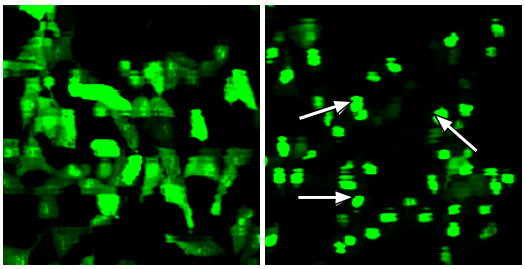

**Figure 1. Nuclear translocation of Rev-EGFP.** Cells expressing Rev-EGFP (DMSO control, left panel) were treated with 50 nM Ratjadone A for 1 hr (right panel). Arrows indicate nuclear accumulation of Rev-EGFP detected by the image analysis algorithm.

## Thermo Scientific Rev nuclear export Redistribution Assay

The human immunodeficiency virus type 1 (HIV-1) regulatory protein, Rev, is a 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].

Nuclear accumulation of Rev can be obtained by specific inhibitors of Crm1-mediated nuclear export such as Ratjadone A [2]. The activity of Ratjadone A in the assay is approximately equivalent to the activity obtained with the natural export inhibitor Leptomycin B. Ratjadone A is used as reference compound in the assay and compounds are tested for their ability to induce nuclear accumulation of Rev. 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 (see related products).

## Features

- Designed to assay compounds for their ability to modulate nuclear export
- 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

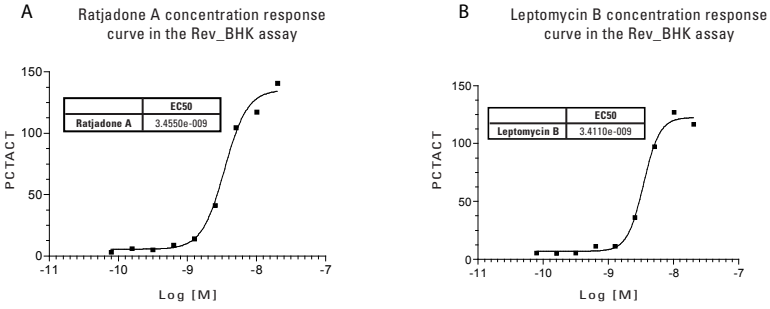


**Figure 2. Concentration response curves in the Rev_BHK assay.** Concentration response was measured in 9 point half log dilution series (n=2). The $EC_{50}$ of both Leptomycin B and Ratjadone A is ~3 nM Cells were incubated with compound for 1 hr. Cells were then fixed and nuclear translocation was measured using the IN Cell Analyzer 3000 (GE Healthcare). % activity was calculated relative to the positive (50 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

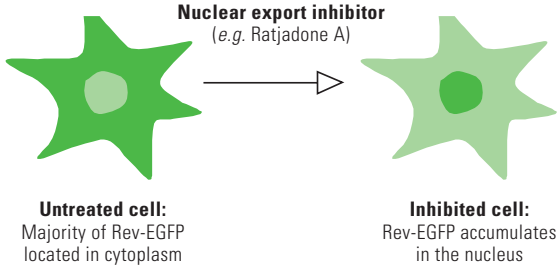


**Figure 3.** Illustration of the Rev translocation event.

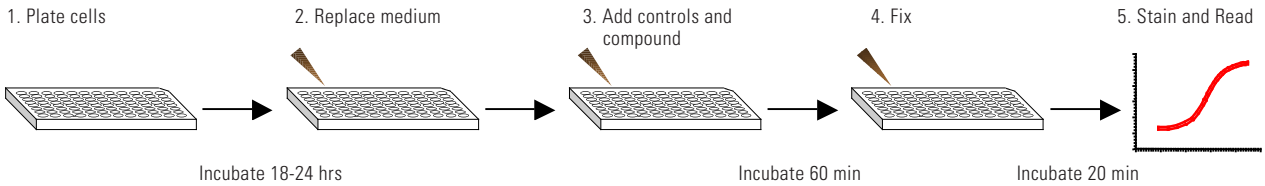


**Figure 4.** The Rev nuclear export Redistribution assay is very easy and fast to perform.

## Thermo Scientific Rev nuclear export Redistribution® Assay

### Assay Details

Recombinant BHK-21 cells stably expressing Rev fused to the N-terminus of enhanced green fluorescent protein (EGFP). The Rev Redistribution assay is designed to assay 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. The Rev nuclear export assay is validated with an average Z′ = 0.35 ± 0.04, 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 VTI

This assay has been validated on the Cellomics Arrayscan VTI 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.

## Ordering Information

| PRODUCT # | DESCRIPTION | CELL LINE | PROFILING | SCREENING | CRYOREDI |
|---|---|---|---|---|---|
| 010_01 | Rev nuclear export Redistribution Assay | BHK-21 | • | • | |

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 |
|---|---|---|---|---|---|
| 062_01 | Rev nuclear export Redistribution Assay | U2OS | • | • | |
| 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 | | | |
| 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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