

Thermo Scientific p53-Hdm2 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.

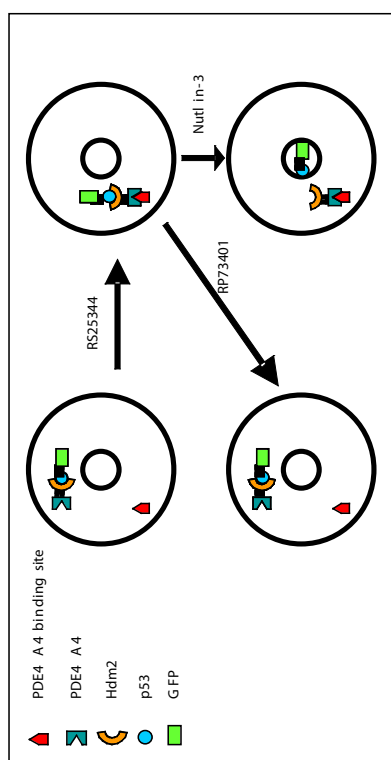


Figure 1. Illustration of the p53-Hdm2 interaction assay.

Thermo Scientific p53-Hdm2 Redistribution Assay

The p53 tumor-suppressor plays a critical role in the prevention of human cancer. In the absence of cellular stress, the p53 protein is maintained at low steady-state levels and exerts very little effect on cell fate. Upon cellular stress, post-translational modifications of p53 cause elevated protein levels and increased transcriptional activity, resulting in cellular changes such as cell cycle arrest, cellular senescence, and apoptosis [1]. The anti-tumor activity of p53 is controlled by its negative regulator, Hdm2, through a feedback mechanism. Hdm2, which is overproduced in many tumors, binds p53 and inhibits its function by modulating its transcriptional activity and stability. Activation of p53 in tumor cells by inhibiting its physical interaction with Hdm2 is therefore a focus of cancer drug discovery.

GRIP technology

The GRIP technology can be used to screen for inhibitors of protein-protein interactions. The technology is based on translocation of the human cAMP phosphodiesterase PDE4A4 [2] to distinct cytoplasmic foci. In general, a bait protein (in this case Hdm2)

is fused to the cAMP phosphodiesterase isoform PDE4A4B, which acts as an inducible anchor protein, and the prey protein (p53) is labelled with GFP. Figure 1 illustrates the configuration of the assay system: Treatment with the PDE4A4 Redistribution agonist RS25344 [3] leads to localization of PDE4A4 into compact foci, and through the interaction between p53 and Hdm2, the GFP tag (on p53) is recruited to PDE4A4 binding sites, resulting in GFP-labeled foci. The PDE4A4 Redistribution antagonist RP73401 [4] antagonizes the effect of RS25344 causing focus dispersal, thereby serving as a universal reference compound for the assay system.

Features

- Designed to assay compounds for their ability to modulate p53-Hdm2 protein-protein interaction
- 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

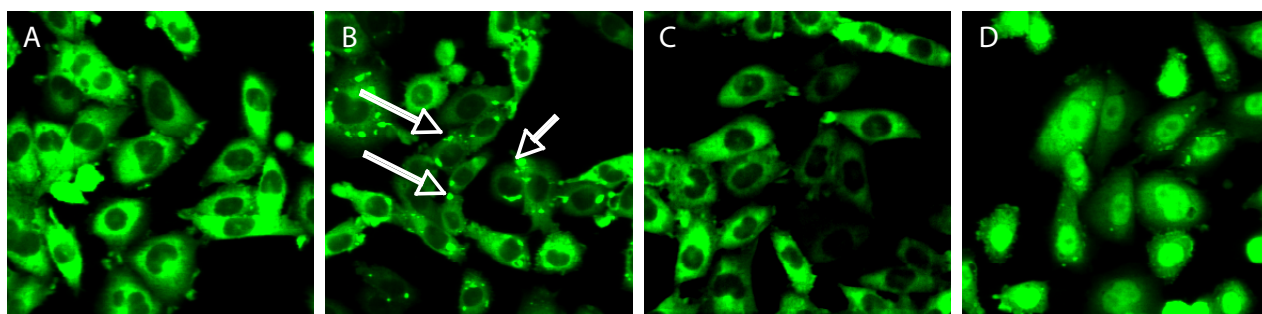


Figure 2. Representative images illustrating that RS25344-induced foci are dispersed by Nutlin-3 and RP73401 in the p53-Hdm2 Redistribution assay. A) Vehicle control (0.25 % DMSO), B) 1 μ M RS25344, C) 1 μ M RS25344 followed by 10 μ M RP73401, and D) 1 μ M RS25344 followed by 10 μ M Nutlin-3. The images were acquired from cells seeded in the presence of 1 μ M RS25344 to induce foci formation (marked by arrows). After 24 hours incubation the cells were treated with 10 μ M RP73401 or 10 μ M Nutlin-3 for 2 hours.

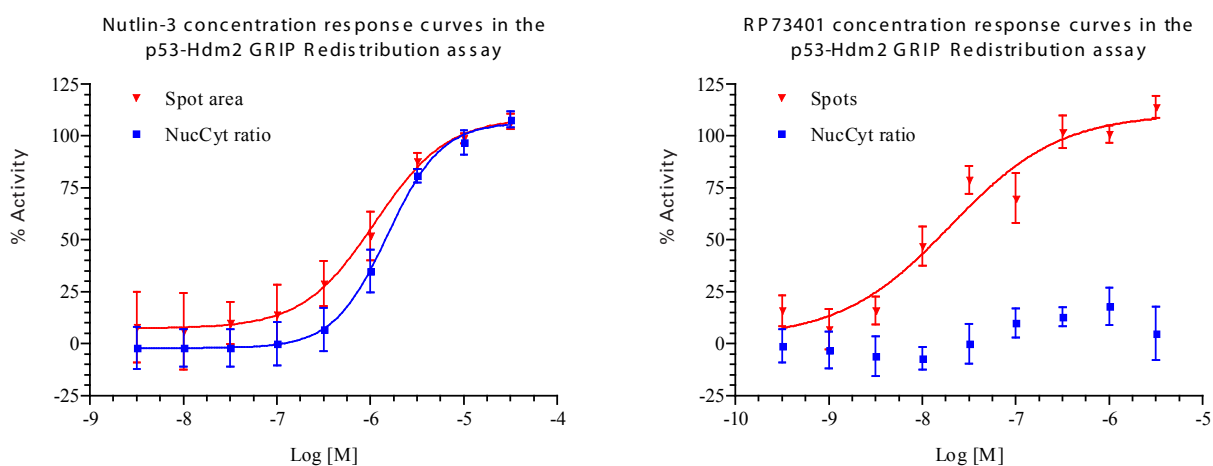


Figure 3. Concentration response curves in the p53-Hdm2 GRIP Redistribution assay. A) Nutlin-3 concentration response (n=16). The response was measured using the Cellomics ArrayScan V^{Hi} Reader and the Redistribution V3 or the SpotDetector V3 BioApplications. The EC₅₀ value of Nutlin-3 is ~1.5 μ M both when using nuclear translocation and spot dispersal as readouts. B) RP73401 concentration response (n=5). The response was measured using the In Cell Analyzer 3000 and algorithms measuring nuclear translocation and granularity. The EC₅₀ value of RP73401 is ~20 nM when using granularity as readout. No nuclear translocation is observed with this compound. Concentration response was measured in 9 point half log dilution series. Cells were pre-incubated with 1 μ M RS25344 for 18-24 hours and treated with test compound for 2 hrs. Cells were then fixed and translocation was measured using the Cellomics ArrayScan V^{Hi} Reader (A) or the In Cell Analyzer 3000 (B). % activity was calculated relative to the positive (10 μ M Nutlin-3) and negative control (0.25% DMSO).

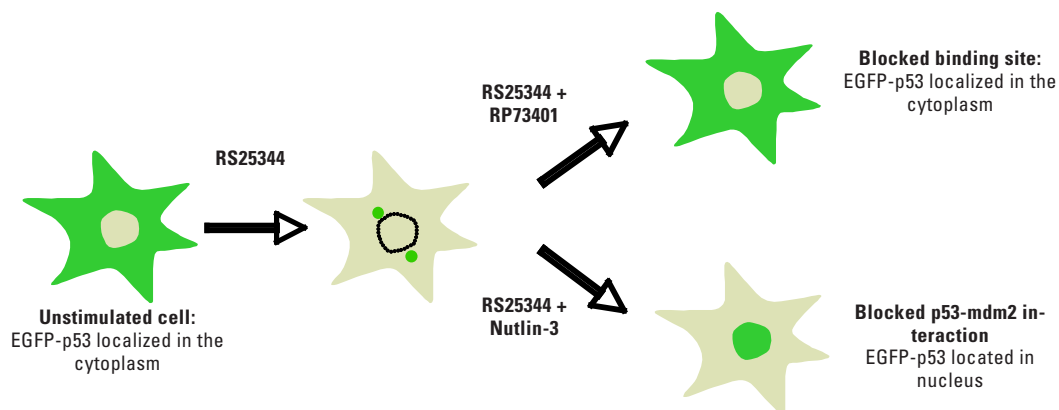


Figure 4. Illustration of the p53 translocation event.

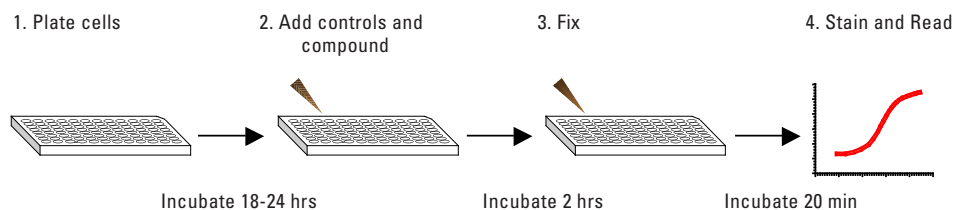


Figure 5: The p53-Hdm2 Redistribution assay is very easy and fast to perform.

Thermo Scientific p53-Hdm2 Redistribution® Assay

Assay Details

Recombinant CHOhr cells stably expressing human p53(1-312) fused to the C-terminus of enhanced green fluorescent protein (EGFP) and PDE4A4-Hdm2(1-124), a fusion protein between PDE4A4 and Hdm2(1-124). The p53-Hdm2 GRIP Redistribution assay is designed to measure the interaction between p53 and Hdm2. Nutlin-3 is a small molecule p53-Hdm2 interaction inhibitor developed by rational drug design [5], and serves as reference compound. Nutlin-3 causes loss of GFP fluorescence from the PDE4A4 foci (Figure 1) by dissociating the interaction between PDE4A4-Hdm2[1-124] and EGFP-p53[1-312]. In addition, Nutlin-3 treatment results in a significant increase in nuclear fluorescence due to nuclear translocation of EGFP-p53[1-312]. This phenomenon is not observed for RP73401 directed focus dispersal, and indicates that unbound EGFP-p53[1-312] is available to the nuclear translocation machinery of the cell. The differential localization of EGFP-p53 and p53 in complex with PDE4A4-Hdm2 allows separation of two types of compounds in this assay: p53-Hdm2 interactors (Nutlin-like) and PDE4A4 dislocators (RP73401-like false positives). Nutlin-3 has an EC_{50} of $\sim 1.5 \mu\text{M}$ in the assay, and compounds are assayed for their ability to inhibit the p53-Hdm2 interaction. The p53-Hdm2 assay is validated with an average $Z' = 0.63 \pm 0.01$ for the nucleus to cytoplasm translocation and an average $Z' = 0.50 \pm 0.15$ for the spot dissociation, suitable for both screening and profiling applications.

Imaging

The translocation of p53/Hdm2 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 outputs in the p53-Hdm2 Redistribution assay are the dissociation of spots in the cytoplasm and the translocation of EGFP-p53 from the cytoplasm to the nucleus. The data analysis should therefore report an output that corresponds to number, area, or intensity of spots in the cytoplasm and an output relating to the GFP fluorescence intensities in the nucleus and the cytoplasm.

Imaging on Thermo Scientific Cellomics ArrayScan V^{TI}

This assay has been validated on the Cellomics Arrayscan V^{TI} using a 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and both the SpotDetectorV3 BioApplication and the RedistributionV3 BioApplication. The output parameter used in SpotDetectorV3 was SpotTotalAreaPerObject; in RedistributionV3 the output parameter 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 Translocation V2, Compartmental AnalysisV2, NucTransV2, and ColocalizationV3.

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
020_01	P53-Hdm2 Redistribution Assay	CHO	•	•	

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
029_01	PDE4A4 Redistribution Assay	CHO	•	•	
8400501	Cellomics Phospho-p53 and p53 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8400601	Cellomics p53 and p21 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8401801	Cellomics MDM2 and p53 Detection HCS Reagent Kit	Antibody- and dye-based reagent kit			
8405701	Cellomics Phospho-ATM and p53 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8402801	Cellomics Phospho-Chk2 Activation Kit 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

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