

Thermo Scientific Rad51 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.

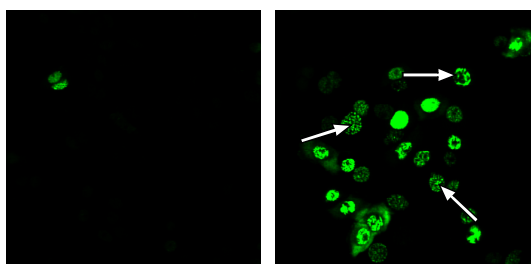


Figure 1. Accumulation of Rad51 in nuclear foci after DNA damage induced by camptothecin. Rad51_SW480 cells were treated with 0.25% DMSO (vehicle control, left panel) or 1 μ M camptothecin (right panel). Arrows indicate camptothecin-mediated nuclear accumulation of EGFP-Rad51 detected by the image analysis algorithm.

Thermo Scientific Rad51 Redistribution Assay

DNA damage causes chromosomal instability which leads to oncogenesis, apoptosis, and severe failure of cell functions. The DNA repair system includes base excision repair, nucleotide excision repair, mismatch repair, translesion replication, non-homologous end-joining, and recombinational repair. Rad51 plays a central role in homologous recombination repair mechanisms that are induced after replication-associated DNA double-strand breaks. Formation of replication-associated DNA double-strand breaks leads to activation of the ATM/ATR kinases, which then phosphorylate and activate the checkpoint kinase Chk1. It is believed that activated Chk1 promotes the association of Rad51 with chromatin, thereby leading to amplification of Rad51 in nuclear foci (Rad51 foci) containing proteins involved in homologous recombination repair [1].

Features

- Designed to assay compounds for their ability to modulate amplification of Rad51 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

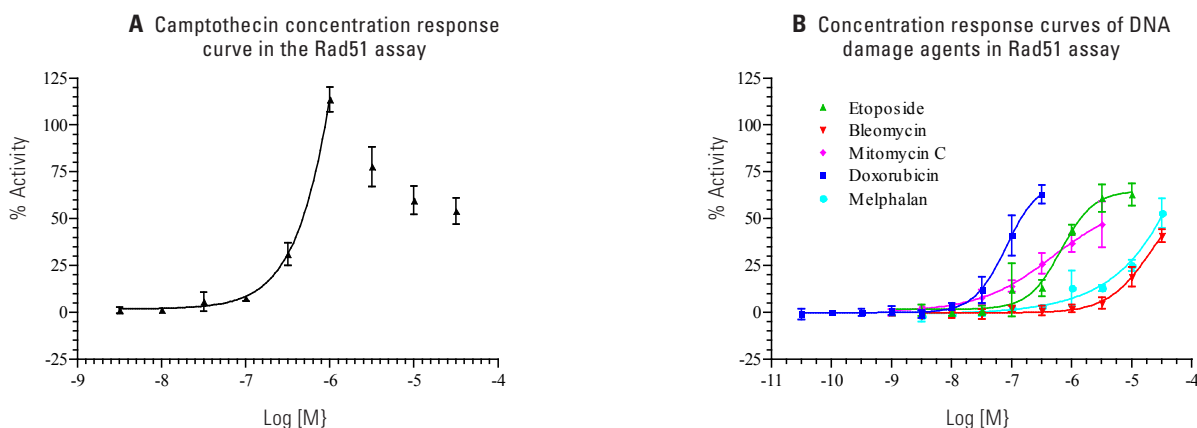


Figure 2: Concentration response curves in the Rad51 Redistribution assay. Concentration response was measured in 9 point half log dilution series of test compounds. Cells were then fixed and the nuclear accumulation of EGFP-Rad51 was measured by image analysis. PCTACT (= % activity) was calculated relative to the positive (camptothecin) and negative control (0.25% DMSO). A) Concentration response curve of positive control compound camptothecin. The EC_{50} value of camptothecin is $\sim 0.5 \mu\text{M}$. B) Concentration response curves of DNA damage inducing agents. EC_{50} values are: Doxorubicin 80 nM, Etoposide 670 nM, Mitomycin C 500 nM, Bleocin and Melphalan $>10 \mu\text{M}$.

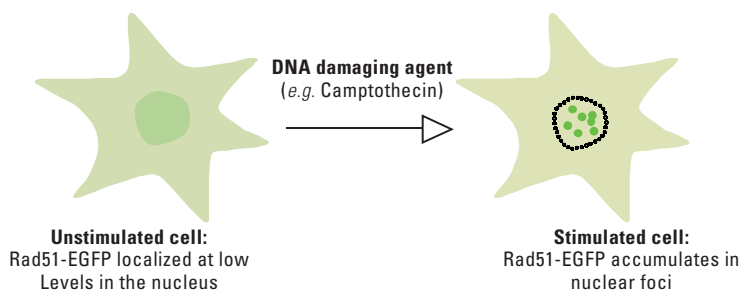


Figure 3. Illustration of the Rad51 translocation event.

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Assay Details

The assay is based on recombinant SW480 cells stably expressing human Rad51 fused to the C-terminus of enhanced green fluorescent protein (EGFP). The Rad51 Redistribution assay is designed to assay for compounds inducing replication-associated DNA double-stranded breaks, since such compounds lead to accumulation of the Rad51-GFP fusion protein in nuclear foci. Replication-associated DNA double-strand breaks is promoted by a wide range of classical DNA damaging compounds, including camptothecin, actinomycin D, doxorubicin, and hydroxyurea [2,3,4]. Camptothecin is used as reference compound in the assay. The Rad51 assay is validated with an average $Z' = 0.73 \pm 0.10$, suitable for both screening and profiling applications.

Imaging

The translocation of Rad51 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 Rad51 Redistribution assay is the increase in nuclear intensity of EGFP-Rad51 and formation of spots in the nucleus. The data analysis should therefore report an output that corresponds to the nucleus intensity or the 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 10x objective (0.63X coupler), XF100 filter sets for Hoechst and FITC, and the RedistributionV3 BioApplication. The output parameter used was MEAN_CircAvgInten. The minimally acceptable number of cells used for image analysis in each well was set to 400 cells. Other BioApplications that can be used for this assay include MolecularTranslocation V2, CompartmentalAnalysisV2, SpotDetectorV3 BioApplication, NucTrans and ColocalizationV3.

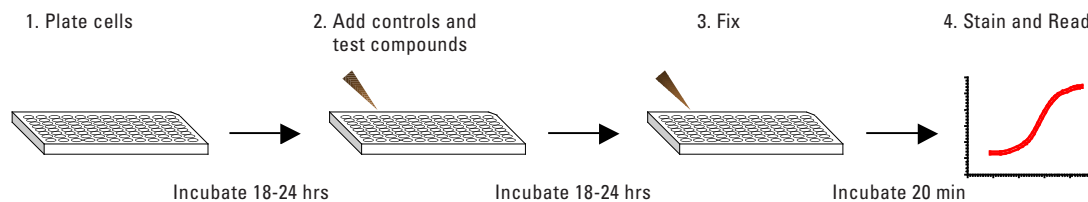


Figure 4. The Rad51 Redistribution assay is very easy and fast to perform

Ordering Information

PRODUCT #	DESCRIPTION	CELL LINE	PROFILING	SCREENING	CRYOREDI
042_01	Rad51 Redistribution Assay	SW480	•	•	•

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
077_01	PXR Redistribution Assay	U2OS	•	•	•
K11-0001-1	Cellomics Micronucleus HCS Reagent Kit	Antibody- and dye-based reagent kit			
8403001	Cellomics Phospho-ATM Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8402801	Cellomics Phospho-Chk2 Activation HCS Reagent Kit	Antibody- and dye-based reagent kit			
8402901	Cellomics Phospho-H2AX 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

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2. Hsiang YH et al., *J Biol Chem.* 260, 14873-14878, 1985.
3. Abe H et al., *Anticancer Res.* 14, 1807-1810, 1994.
4. Yarbro JW, *Semin Oncol. Biol. Chem.* 19, 1-10, 1992.

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