

## The Rad51 Redistribution<sup>®</sup> Assay

DNA damage causes chromosomal instability leading 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 that in turn 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].

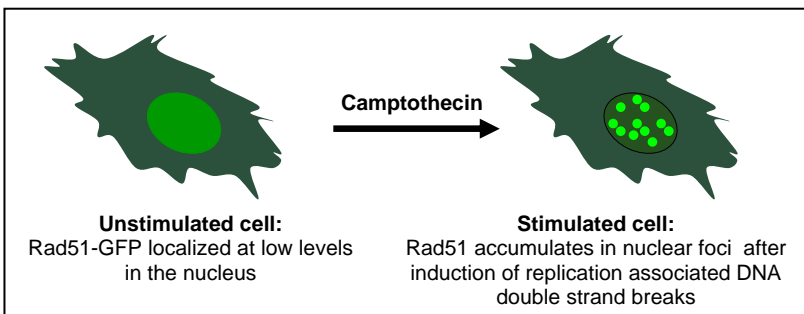


Figure 1: Illustration of the Rad51 translocation event.

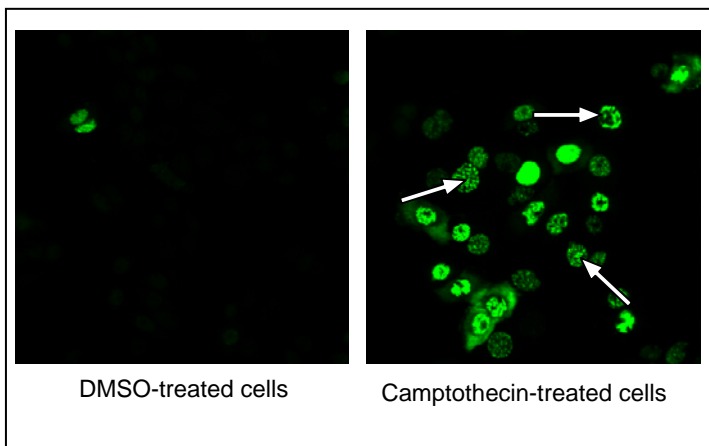


Figure 2: Images illustrating cells treated in the absence (DMSO) or presence of camptothecin. Arrows indicate the accumulation of Rad51-GFP in the nuclei of camptothecin-treated cells detected by the image analysis algorithm.

The Rad51 Redistribution<sup>®</sup> Assay is designed to assay for compounds inducing replication-associated DNA double stranded breaks as such compounds lead to accumulation of the Rad51-GFP fusion protein in foci within the nucleus. 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 having an EC<sub>50</sub> value of ~ 140 nM.

Test compounds causing nuclear foci-formation of Rad51 can be considered as DNA damaging agents that directly or eventually indirectly generate replication-associated DNA double stranded breaks.

Figures 1 and 2 illustrate the translocation event and Figure 3 illustrates the timeline involved in the Rad51 assay. Cells are incubated for 18-24 hours with compound in serum-containing medium. The cells are fixed and stained with a nuclear stain before the assay response is read in the IN Cell 3000 Analyzer (GE Healthcare). Compounds that induce replication-associated DNA double stranded breaks, and thereby give rise to Rad51 foci in the nucleus, are positive in the assay. The degree of DNA damage is calculated as percent activity (PCTACT) relative to the camptothecin control.

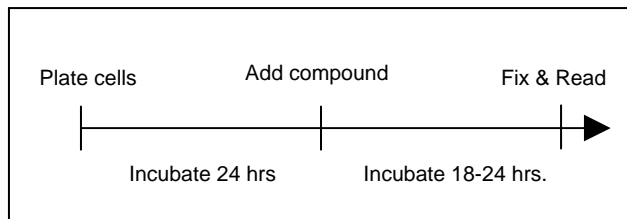


Figure 3: Summary of the assay timeline.

## Assay Details

Cell line: SW480 PS2141 GS Cl. 1B6 (Rad51-GFP) seeded in Dulbecco's Modified Eagle Medium (DMEM) with Glutamax-1 containing 10 %  $V/V$  FBS, 1%  $V/V$  Penicillin-Streptomycin, and Geneticin 0.5 mg/ml. Table 1 shows the final concentrations of DMSO, serum and camptothecin in the assay.

Assay	DMSO (%)	Serum (%)	Camptothecin ( $\mu\text{M}$ )
Rad51	0.25	5	1

Table 1: Final DMSO, serum, and camptothecin concentrations.

## Test compound handling for profiling services

Test compounds are stored at 4°C until diluted in neat DMSO. DMSO solutions are stored at -20°C. For preparation of the master concentration-response plates, neat DMSO is added to each compound to give a final compound concentration of 25 mM. The master concentration-response plates are generated in half log dilutions corresponding to concentration response curves in the range 3.16 nM-31.6  $\mu\text{M}$ .

## Concentration response curve of the assay reference compounds

Figure 4A shows concentration response curves of the reference compound camptothecin. The  $\text{EC}_{50}$ -value of camptothecin is in the nano-molar range (*i.e.* ~140 nM). The DNA damaging agents doxorubicin [3], bleocin [3], etoposide [5], melphalan [6,7], and mitomycin C [8] all have activity in the Rad51 assay (Figure 4B).

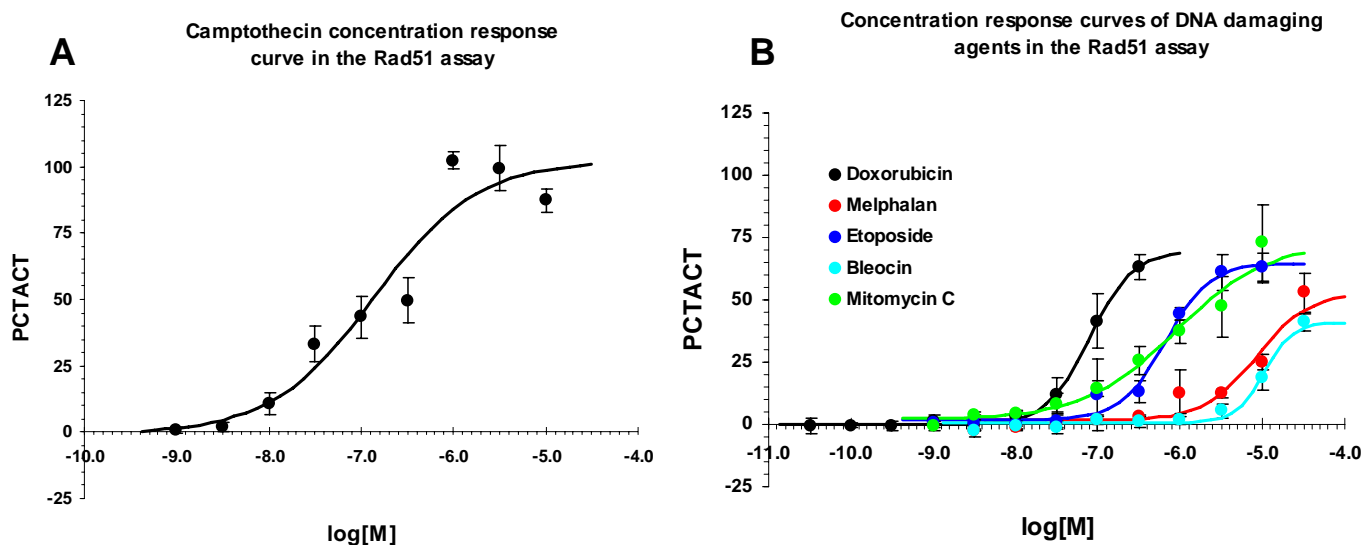


Figure 4: **A**) Camptothecin (n=4) concentration response curve. **B**) Concentration response curves of various DNA damaging agents (n=4).

## References:

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3. Abe H *et al.*, *Anticancer Res.* 14, 1807-1810, 1994.
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## Notes:

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