

### The FKHL1 Redistribution<sup>®</sup> Assay

The mammalian transcription factors FKHR, FKHL1, and AFX function as key regulators of insulin signaling, cell cycle progression and apoptosis downstream of phosphoinositide 3-kinase (PI3K). Forkhead proteins function as classical transcriptional activators, and target genes include pro-apoptotic proteins (e.g. Fas Ligand and Bim) and cell cycle inhibitors (e.g. p27, p130 and GADD45). In growing cells, Forkheads are kept inactive through Akt-mediated phosphorylation of three conserved threonines. Phosphorylation of the threonines causes binding to 14-3-3 proteins in the nucleus followed by nuclear export and cytoplasmic retention.

Furthermore, nuclear export of Forkhead proteins is dependent on the classical NES/Crm1 pathway [1]. Inactive FKHL1 is cytoplasmic but is rapidly imported to the nucleus upon inactivation of the PI3K/Akt pathway. The FKHL1 Redistribution<sup>®</sup> assay is designed to assay for inducers of FKHL1 translocation by monitoring the translocation of a FKHL1-GFP fusion protein from the cytoplasm to the nucleus.

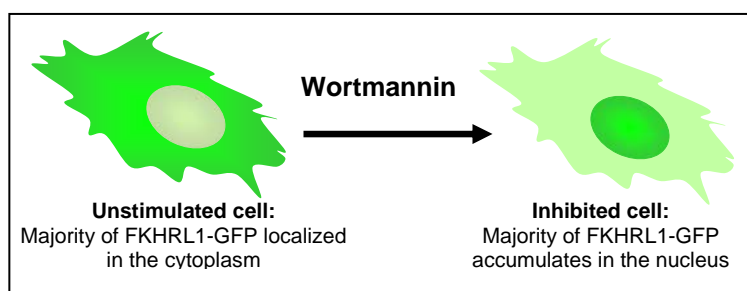


Figure 1: Illustration of the FKHL1 translocation event.

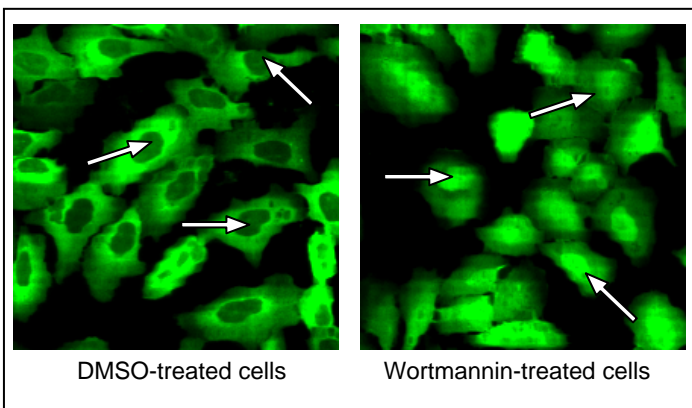


Figure 2: Images illustrating cells treated in the absence (DMSO) or presence of wortmannin. Arrows indicate the cytoplasm-to-nucleus translocation detected by the image analysis algorithm (*i.e.* empty nuclei in DMSO-treated cells and the accumulation of FKHL1-GFP in the nuclei of wortmannin-treated cells).

Compounds inducing nuclear accumulation of FKHL1 can be analyzed for upstream effects using the Akt1 Redistribution<sup>®</sup> assay [4], general export inhibitor characteristics using the Rev1 Redistribution<sup>®</sup> assay [5], or for isoform selectivity using the FKHR Redistribution<sup>®</sup> assay [6]. The FKHL1 Redistribution<sup>®</sup> Assay can also be used to detect compounds acting as general nuclear export inhibitors; in this case the export inhibitors Leptomycin B or Ratjadone A should be used as the reference compound. Contact us for further details.

Figures 1 and 2 illustrate the translocation event, and Figure 3 illustrates the timelines involved in the FKHL1 assay. Cells are incubated for 60 min. with compound in low-serum (1%)-containing medium. The cells are fixed and stained with a nuclear stain before the assay response is read in the INCell 3000 Analyzer (GE Healthcare). Compounds that induce nuclear accumulation of FKHL1 are positive in the assay, and the degree of inhibition is calculated as percent activity (PCTACT) relative to the wortmannin control.

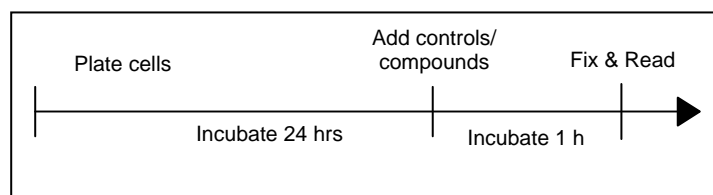


Figure 3: Summary of the assay timeline.

## Assay Details

Cell line: FKHRL1\_U2OS (#C009B) cells expressing FKHRL1-EGFP are grown in Dulbecco's Modified Eagle Medium (DMEM) with Glutamax-1 containing 1.0 mg/ml Geneticin (G418), 1% (v/v) Penicillin/Streptomycin, and 10% FBS.

| Assay  | DMSO (%) | Serum (%) | Wortmannin (nM) |
|--------|----------|-----------|-----------------|
| FKHRL1 | 0.25%    | 1.0%      | 300             |

Table 1: Final DMSO, serum, and wortmannin 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 µM.

## Concentration response curve of the assay reference compound wortmannin

Figure 4 shows concentration response curves of the reference compounds wortmannin and LY294,002 in the FKHRL1 assay. The EC<sub>50</sub>-value of wortmannin is in the low nano-molar range (*i.e.* ~8 nM), whereas the EC<sub>50</sub> of LY294,002 is ~ 3.5 µM.

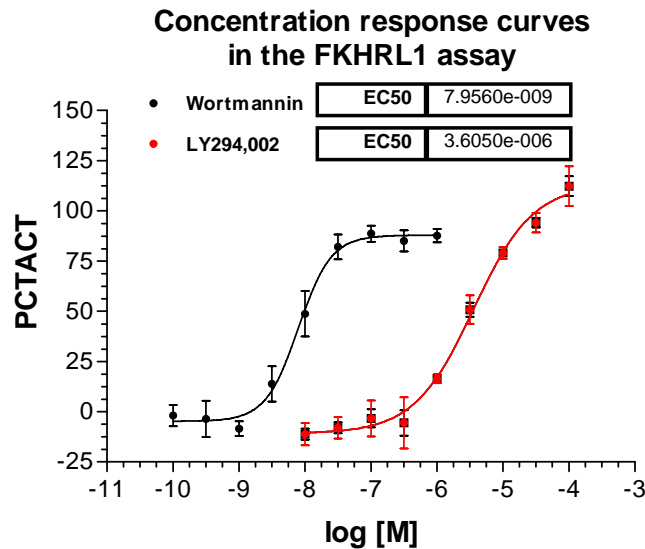


Figure 4: Wortmannin and LY294,002 concentration response curves, n=4.

## References:

- Burgering BMT. & Kops GJPL. *Trends Biochem. Sci.* 27, 352-360, 2002.
- Acaro A. & Wymann MP. *Biochem J.* 296, 297-301, 1993.
- Burgering BM. & Coffey PJ. *Nature* 376, 599-602, 1995.
- BiolImage, *Akt1 Assay Application Note* 07-04-001.
- BiolImage, *Rev1 Assay Application Note* 07-04-010.
- BiolImage, *FKHR Assay Application Note* 07-04-008.

## Notes:

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