

## Assay Name: p53 and phospho-p53 fluorescent marker analysis

**Assay ID:** Celigo\_02\_0009

**Description:** Quantification of p53 and phospho-p53 levels in NCI-H460 cells post Hydroxyurea treatment

**Stains:** CellTracker™ Blue CMAC, secondary Alexa Fluor® 488 antibody, secondary Alexa Fluor® 568 antibody

**Imaging channels:** Green, Red and Blue

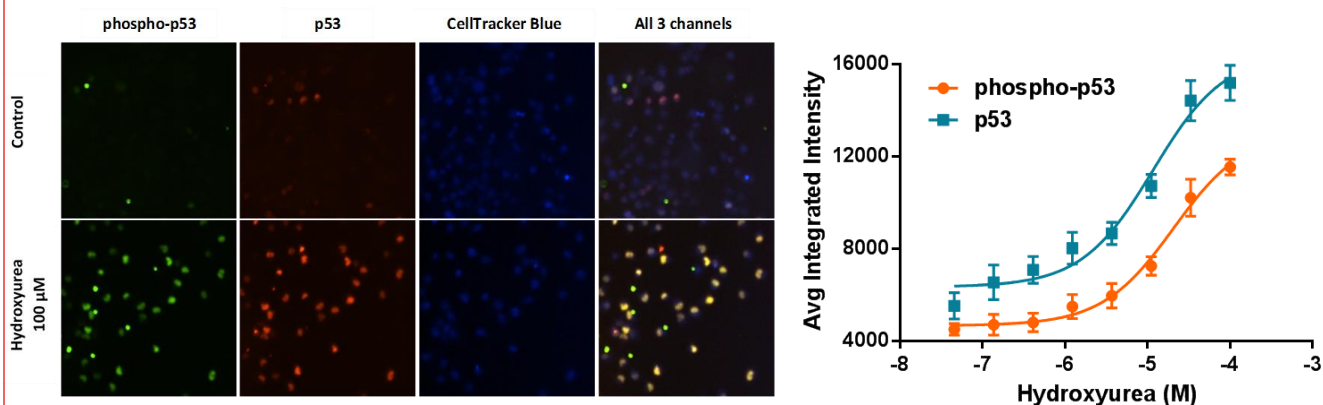
**Image analysis algorithm:** Celigo software Expression Analysis: Target 1 + 2 + Mask

### Methods:

1. Stain cells in the tissue culture flask with CellTracker™ Blue (10  $\mu$ M final), incubate for 30 min at RT
2. Seed 2,000 cells/well in 384-well plate
3. Add desired drug (Hydroxyurea) or control solution in each well and incubate cells for 24h (or 48h) at 5% CO<sub>2</sub>, 37°C
4. Fix and permeabilize the cells
5. Add p53 antibody and phospho-p53 primary antibodies (1:200) and incubate at 4°C overnight
6. Wash plate multiple times and add 10  $\mu$ l of secondary antibody (1:200) and Incubate for 1 hour at RT
7. Image the plate on the Celigo

### Results:

Detection of phospho-p53 and p-53 detection in NCI-H460 cells post Hydroxyurea treatment



- Hydroxyurea is an inducer of p53 upregulation and phosphorylation
- As hydroxyurea concentration increases, we observe an increase in p53 and phospho-p53 signal, and this is depicted in both the image and graphical data above.