



Operetta CLS Demo Information

For testing your own samples:

- ▶ It is usually a good idea, to test different cell numbers (a confluence of 70% is usually best for 2D experiments).
- ▶ Please let us know which excitation and emission wavelength you need for your dyes. This way we can check beforehand how good they will match the Operetta CLS.
- ▶ Please use a nuclear stain (Hoechst, DAPI, Draq5 or similar) for robust segmentation results / assay independent cell identification.
- ▶ Please make sure your samples comply with biosafety rules.
- ▶ If you have compound/ concentration information available as excel file (plate format), they can be used in our Harmony software as well.

- ▶ **Important:** In order to have the full flexibility in objective lens choice,
Use Plates with **thin plate bottoms** (ca. 190µm glass or plastic)
Use the **inner wells of the plate** (skip first or last row or column)
If you use a high bottom 384 well plate (skirt height $H > 1$ mm) use only
the inner wells from C3 to N22

HCS Demonstration – Sample Information

Name/ Group Achim Kirsch – PerkinElmer FAS	Assay name Demecolcine	Plate 384 PerkinElmer CellCarrier (part No. 6007551)
Sample Cell lines, seeding density, fluorophores, targets HeLa cells stained with Alexa488 (tubulin), Alexa647 (pHH3), TRITC (actin) and DAPI. 5000 cells per Well		Plate Layout: Controls, variations (e.g. dose responses) Dose Response of Demecolcine in triplicates (C5 - E13). Negative Control in C4 E4
Measurement Conditions Objective, environment, fields/kinetics 20x or 40x, ambient (no temperature/CO2 control), 4 fields		
Phenotypes or changes to be measured Influence on cell cycle and cell morphology.	Specific system features of interest Image Quality, setting up an analysis	
How is the assay currently evaluated Microscope and scoring of cells with Fiji.		
What improvements are you looking for over your current methodologies?		

Example

HCS Demonstration – Sample Information

Name/ Group	Assay name	Plate
Sample Cell lines, seeding density, fluorophores, targets		Plate Layout: Controls, variations (e.g. dose responses)
Measurement Conditions Objective, environment, fields/kinetics		
Phenotypes or changes to be measured	Specific system features of interest	
How is the assay currently evaluated		
What improvements are you looking for over your current methodologies? 1. 2. 3.		