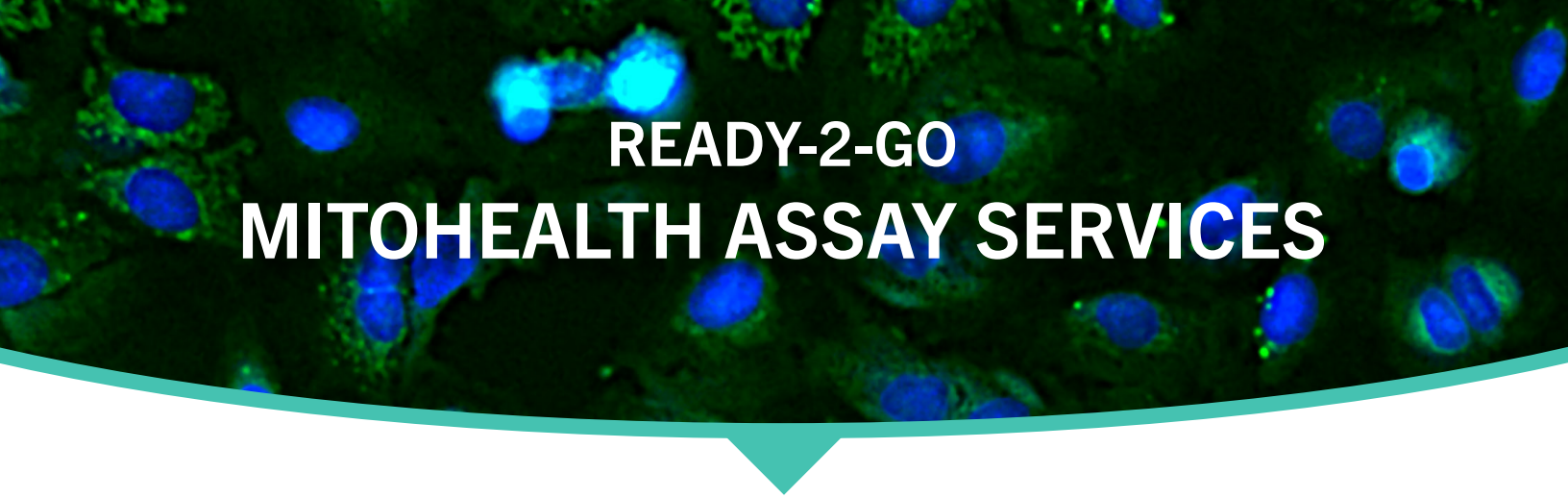


READY-2-GO
MITOHEALTH ASSAY SERVICES

BACKGROUND

Mitochondria play a fundamental role in maintaining cellular health by converting sugars and oxygen into energy (ATP), using a process that relies on the membrane potential of mitochondria. Perturbations in mitochondrial function substantially impact cell health and, therefore, are critical to monitor in any drug development campaign, whether desirable (e.g. in cancer treatment) or not (e.g. off target toxicity). Additionally, drug toxicity must be assessed with any drug candidate prior to clinical trial, rendering assessment of mitochondrial health a valuable tool as an early indicator of toxicity.

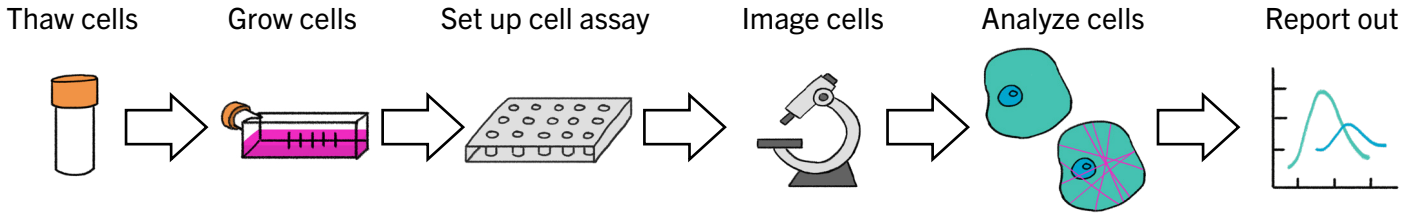
OUR SOLUTION

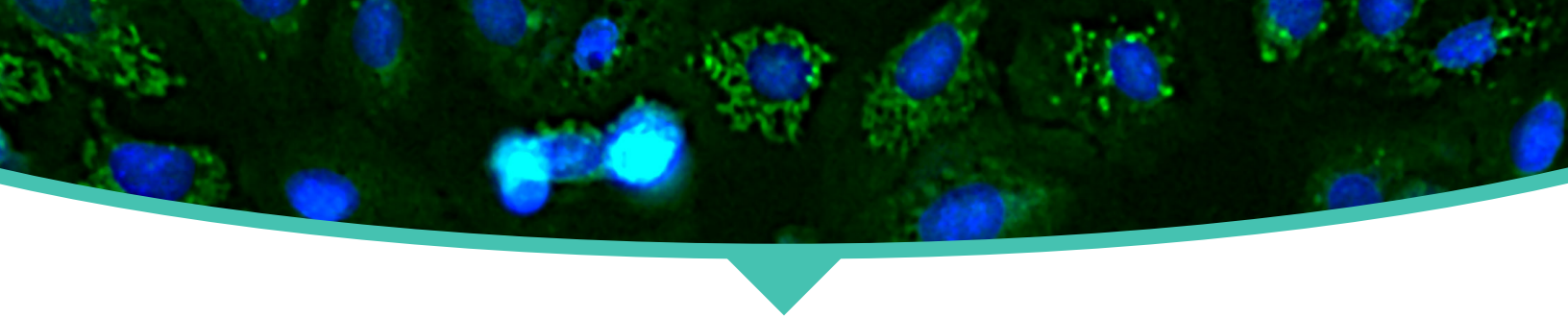
Our Ready-2-Go (R2G) Mitochondrial Health Assay Service screens for changes in mitochondrial membrane potential using a fluorescent dye that accumulates within the mitochondria at a rate that depends upon its membrane potential. We measure the fluorescence intensity of mitochondria from images at the single-cell level and then report out the quantitative results at the population-level. This enables us to assess the effects of your drug candidates as a continuum of cell health and to better understand the variability of the response.

KEY FEATURES & BENEFITS

- **More accurate data.** Intensity measured from mitochondria only - not averaged with non-relevant signals from nearby pixels.
- **Our cell assays are developed for imaging.** U-2 OS and A549 cells are well characterized as having flat morphologies, which minimizes out-of-focus light.
- **R2G assay services are a starting point.** Transition to more complex, bespoke assay services with the same service provider.

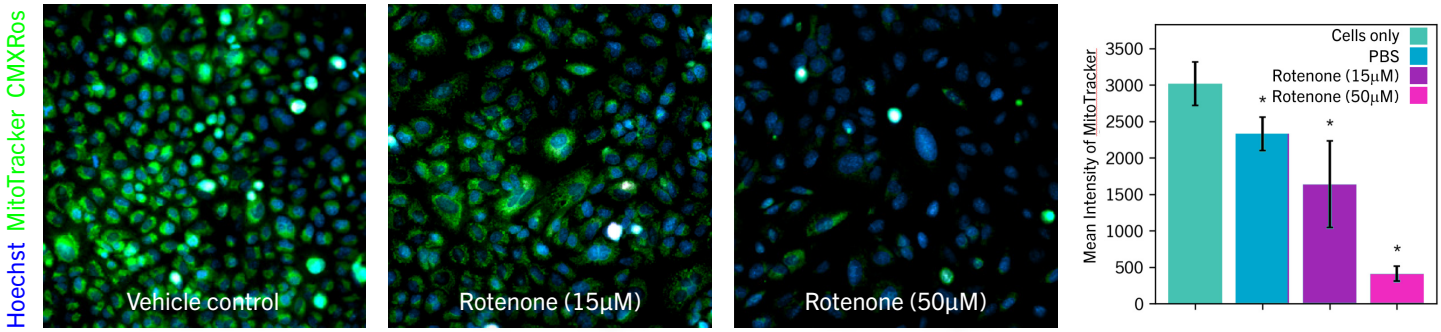
PROCESS OVERVIEW





HOW IT WORKS

HEP G2, A549, or U-2 OS cells are seeded into 384-well plates, allowed to grow, and then incubated in the presence of compound for 4 or 24 hours. After treatment, cells are fixed and stained in preparation for imaging and analysis.



The representative images of A549 cells shown above were captured following 24 hours of treatment with vehicle (left image), 15µM Rotenone (middle), or 50µM Rotenone (right). Mitochondrial staining intensity (shown in green) is indicative of healthy mitochondria and is highest under vehicle control and decreases in a dose-dependent manner upon rotenone treatment, as expected and similar to what we observe for U-2 OS and HEP G2 cells under similar conditions.

ASSAY SERVICE DETAILS

	Ready-2-Go Mitochondrial Health	Bespoke Assay Services
Cells (select one)	A549 (Human lung carcinoma epithelial cells) U-2 OS (Human osteosarcoma epithelial cells) HEP G2 (Human hepatocellular carcinoma cells)	If you would like to expand the service offering beyond R2G shown on left, please contact us at info@phenovista.com or reach out to your local sales representative.
Markers	Hoechst (all nuclei), MitoTracker CMXRos (mitochondria), DRAQ7 (dead nuclei)	
Dosing	6 doses of your test article	
Positive Control(s)*	FCCP, Rotenone, Paraquat	
Format	384-well plate	
Replicates	Triplicates (at a minimum)	
Assay Readouts	Nuclear count MitoTracker intensity Percentage viable cells	

* Vehicle and untreated controls also included.

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