



THE POWER OF

GUAVA MITOPOTENTIAL™ ASSAY:

For monitoring changes in the mitochondrial membrane potential

Guava MitoPotential Assay

- Sensitive, single cell analysis
- No-wash, mix and read assay format
- Easily discern healthy/polarized cells from apoptotic/depolarized and dead cells
- Works with both suspension and adherent cells
- Convenient 96-well or tube format options
- Determine if cells have entered late apoptosis with or without depolarization (7-AAD addition)

Mitochondria biology is one of the fastest growing areas in genetics and medicine, having been implicated in over 100 diseases such as Parkinson's, diabetes, dementia, multiple sclerosis, congestive heart failure, cancer metastases, to name a few. Hence, there is growing interest within the scientific community to evaluate changes in the mitochondrial membrane potential ($\Delta\Psi_m$) and further define its role in initiating apoptosis, cell cycle, or other cellular processes. Loss of the $\Delta\Psi_m$ is often, but not always, observed to be associated with the early stages of apoptosis. Collapse of this potential is believed to coincide with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome C into the cytosol which then triggers the downstream events in the apoptotic cascade.

Automated single-cell analysis is the technique of choice for monitoring the $\Delta\Psi_m$ due to its sensitivity and high reproducibility. However, since traditional methods are hard to use and require specially trained operators, the technique is limited to core flow labs or other population-based methods such as manual microscopy or fluorescence microplate readers. The Guava MitoPotential Assay is a no wash, mix and read assay that easily discerns healthy, polarized cells from apoptotic, depolarized cells. The unique addition of the dead cell dye, 7-AAD, has the added benefit of assessing if the cells have entered late apoptosis with or without depolarization. The assay is more informative than population-based methods and more quantitative than microscopy methods. When used in conjunction with the Guava EasyCyte™ system, the assay provides single-cell

based sensitivity with the ease of use, throughput (96-well option), and convenience of a microplate reader.

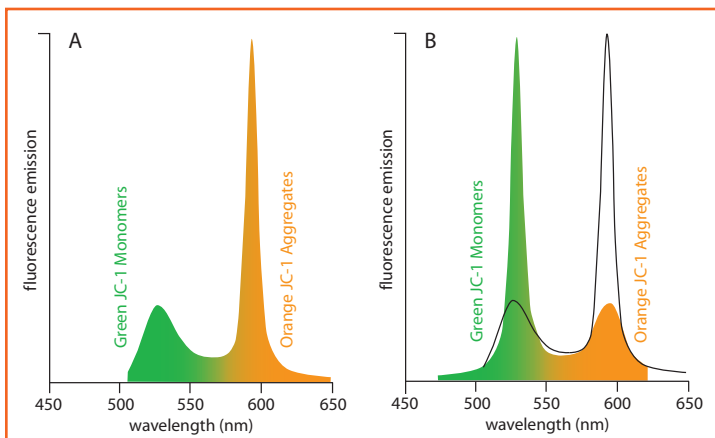


FIGURE 1A: *JC-1 Properties – Healthy Cells:* Healthy cells incubated with JC-1 will fluoresce mostly orange (590 nm-max) as JC-aggregates are formed in the mitochondria due to increases in dye concentration; a small percentage of this dye will also fluoresce green (527 nm-max) as monomers in the cell cytoplasm. FIGURE 1B: *JC-1 Properties – Depolarized/Apoptotic Cells:* As cells undergo apoptosis, holes form in the mitochondrial membrane, which permit the dye to leak into the cell cytoplasm where the dye exists as monomers. This results in the fluorescence of this dye to shift from mostly orange to mostly green.

GUAVA MITOPOTENTIAL ASSAY BENEFITS

No Wash. Mix and Read Assay Format (Figure 2)

Automatically calculates all relevant statistics in a convenient Analysis Results Table (Figure 3)

Comparable in performance to another vendor's kits or alternative apoptotic methods (Figure 4)

Guava Mitopotential Assay

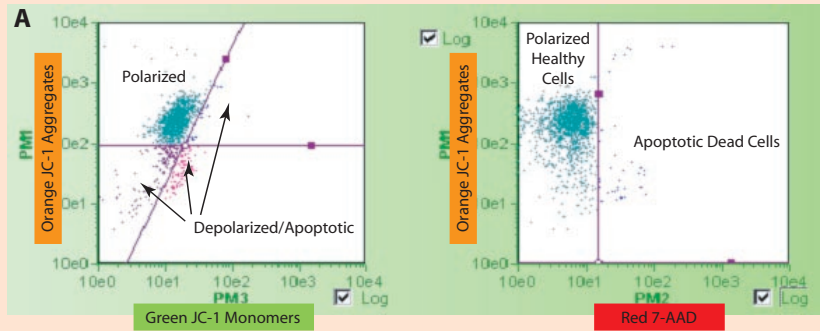
Induce Cells

Add Staining Solution

Incubate 30 minutes

Acquire & Analyze Cells on Guava EasyCyte System

FIGURE 2: No-wash, mix and read assay format.



Analysis Results	Count	Cells/mL	% Total	Grn MFI	Org MFI	Red MFI
Polarized Cells (UL, Plot 2)	1221	1.45e05	81.40%	14.76	284.56	—
Depolarized Cells 1 (UR, Plot 2)	22	2.61e03	1.47%	41.64	307.32	—
Depolarized Cells 2 (LR, Plot 2)	95	1.13e04	6.33%	18.23	39.99	—
Depolarized Cells 3 (LL, Plot 2)	162	1.92e04	10.80%	9.08	54.43	—
Apoptotic/Dead Cells (Right, Plot 3)	38	4.50e03	2.53%	—	680.71	40.50

FIGURE 3: The pattern of JC-1 staining may vary between cell type and cell line. In this example, healthy, uninduced Jurkat cells are ~81% polarized/healthy, ~17% depolarized/apoptotic, and <3% dead (Fig. 3A). The assay statistics are automatically calculated and displayed for easy interpretation of results (Fig. 3B).

MitoPotential Accuracy Data

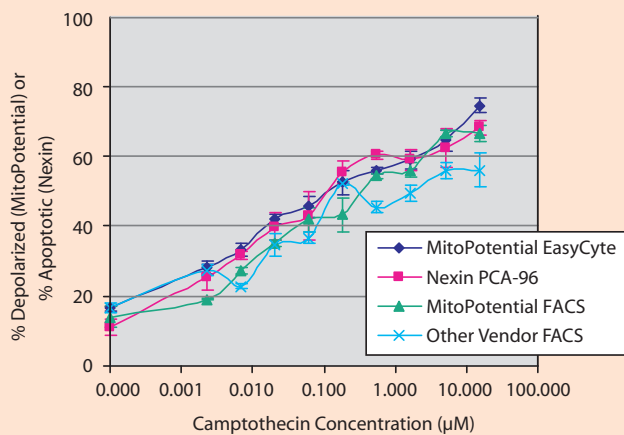


FIGURE 4: HeLa cells were induced overnight with camptothecin at the various concentrations indicated, assayed using the Guava MitoPotential kit, and compared against three other approaches: Guava Nexin™ kit (Annexin-V binding) using the Guava PCA™-96 system; Guava MitoPotential kit analyzed on a FACS; and another vendor's JC-1 kit analyzed on the FACS. Results are comparable using all four approaches.

ORDERING INFORMATION

Guava EasyCyte MitoPotential Kit (100 tests) 4500-0250

Guava EasyCyte MitoPotential Software Module 0500-1230

For pricing, please request a quotation.



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