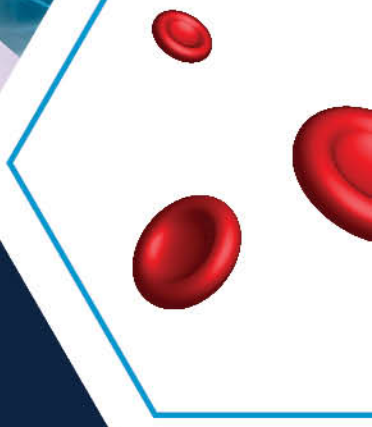
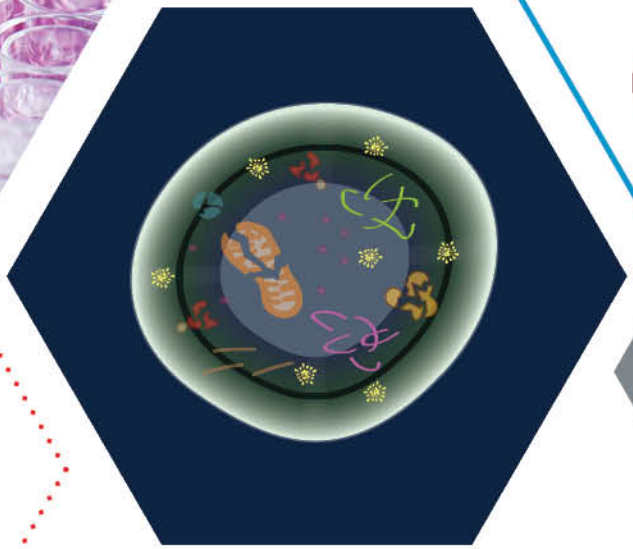
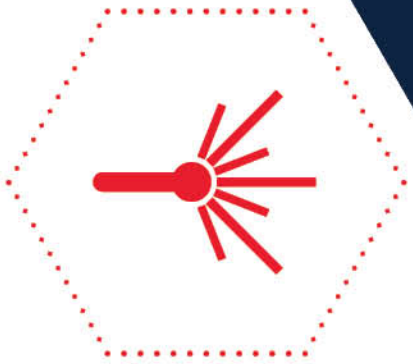
EVERY
event matters.







CELL HEALTH ASSAYS

FOR FLOW CYTOMETRY

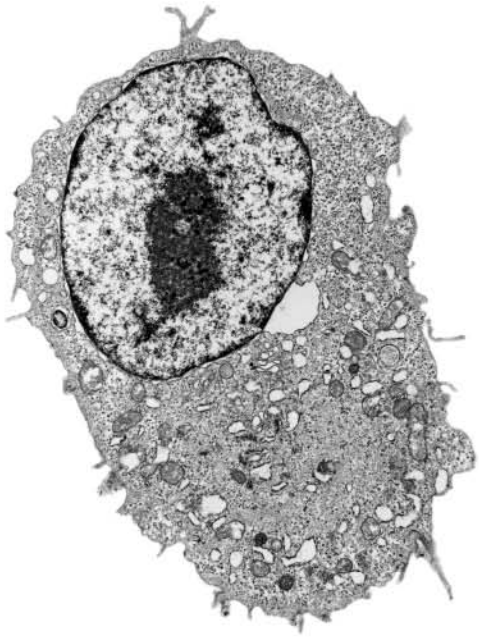
BECKMAN COULTER
Life Sciences

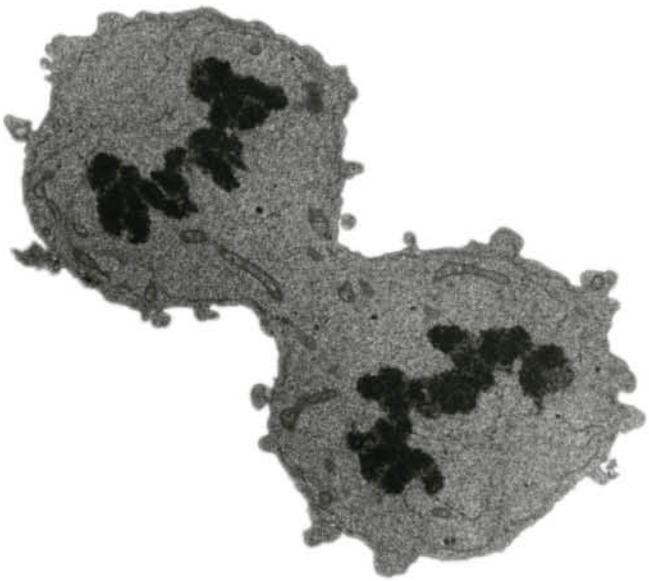
CELL HEALTH

The vitality of the cell is fundamental to its biological function and response. Quantifying cell health is often context dependent. Does the cell maintain proliferative capability? Is the cell metabolically active? Is the cell structure intact? Cell health assays are readouts of only certain of these functions.

Isogenic cells in the same culture environment can behave differently. Analysis at the single cell level gives investigators insight into the biological heterogeneity and can be the key to better understanding the factors affecting a specific biological response. This can be useful in basic research understanding complex systems biology or in applied biosciences such as the effect of drugs or culture conditions.

Beckman.com/cell-health





Quiescent/Senescent

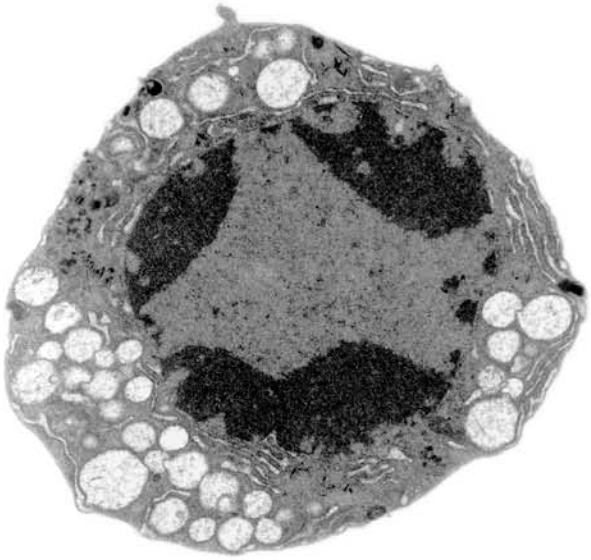
Most prominent state of cells of the body. Cells are not dividing, are intact and metabolically active depending on the cell type. Quiescent cells are able to enter the cell cycle. Senescent cells have lost the reproductive capacity. Assays of cellular function are useful for assessing metabolic state.

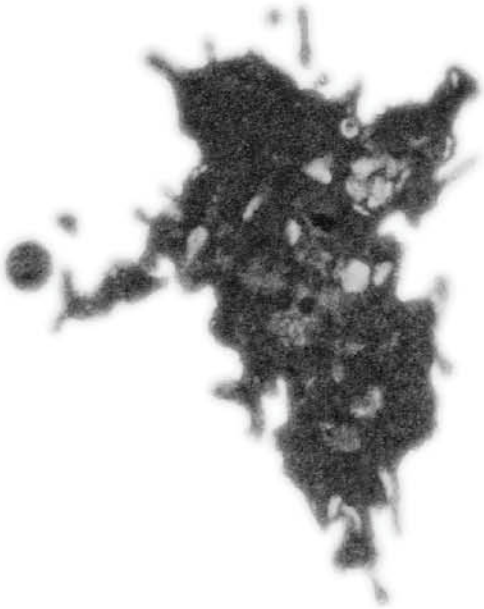
Division

The cell cycle is a tightly controlled. During this process the cell duplicates its genetic material prior to cytokinesis. The process can be monitored by assessing key regulators as well as DNA quantitation.

Cell Viability: the percentage of cells that are living.

Cell Vitality: measurement of the health of the cell. Includes assessment of proliferation capability, ability to complete key functions, absence of programmed cell death signals.





Apoptosis

Organized and controlled cell death process utilized in development and homeostasis. Proceeds through tightly regulated steps which can be detected in various assays, from initiation steps through to terminal effects. The cell membrane is intact. Cells are dying but will stain positive in a viability assay.

Necrosis

Response to mechanical disruption of the membrane, acute toxicity, shock from heat or sudden loss of nutrients. This unregulated cell death is always harmful to the organism. Assays are based on the lack of membrane integrity.

CELL VIABILITY

Cell viability is important in several pathophysiological contexts. Cell death measurements, both as a primary endpoint or in the context of potency metrics such as IC50 and EC50, are used to determine the cytotoxicity of pharmacological agents. This is important for determining optimal dose concentrations, balancing potency with side-effect limitation. It is also key for ascertaining the effectiveness of cytotoxic agents used as cancer therapeutics. In addition, assessing cell viability is an important quality control measure in immunophenotyping. Dead cells bind antibodies non-specifically skewing the percent positive calculations. Dead cells also increase in autofluorescence interfering with detection of specific signals. Gating out dead cells should be completed prior to population analysis by sequential gating.

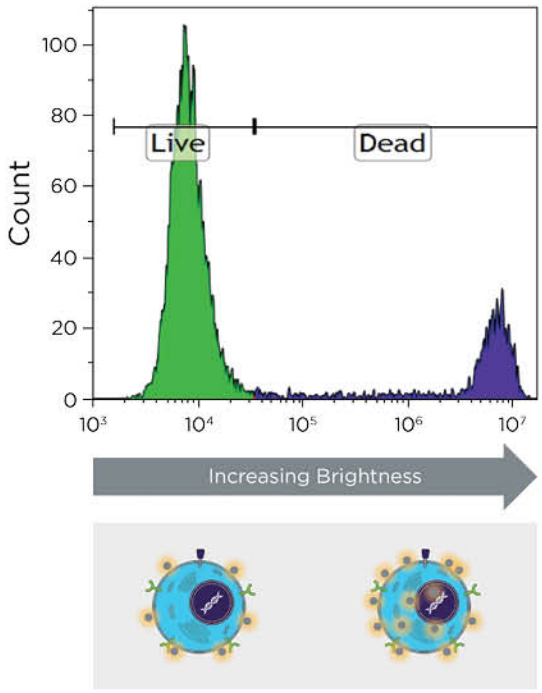
Membrane Impermeant DNA Binding Dyes

Excluded by the intact membranes of live cells. However inside the cell, via diffusion through porous membranes, they bind DNA and fluoresce.

Part Number	Reagent	Size	Excitation Max	Emission Max
B88526	7-AAD	150 tests/ 1 vial	546 nm	647 nm
B25595	DRAQ7	200 tests/1 vial	633 nm	695 nm
B30437	DAPI	200 tests/1 vial	358 nm	461 nm

ViaKrome Fixable Viability Dyes

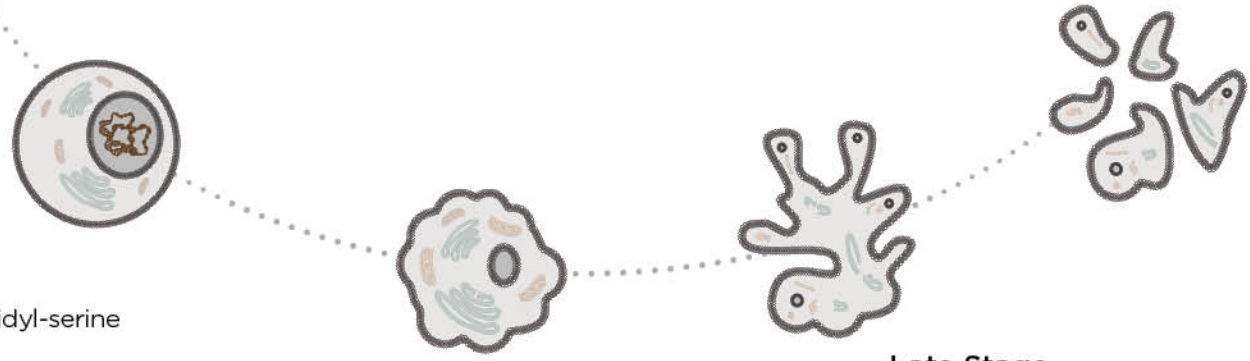
ViaKrome fixable viability dyes bind to reactive thiols. These dyes are cell membrane impermeant. Therefore in living cells the proteins on the cell surface are accessible to take part in the reaction. Cells with compromised membranes will allow the dye to diffuse inside the cell where it can interact with a larger concentration of reactive proteins. This results in dead cells staining brighter than live cells. Furthermore, this staining pattern can be preserved by fixation, a process required for intracellular marker staining.



Part Number	Reagent	Size	Excitation Max	Emission Max
C36614	ViaKrome 405	200 tests/1 vial	401 nm	420 nm
C36620	ViaKrome 561	200 tests/1 vial	555 nm	565 nm
C36624	ViaKrome 638	200 tests/1 vial	638 nm	655 nm
C36628	ViaKrome 808	200 tests/1 vial	854 nm	878 nm

APOPTOSIS

In early apoptosis, overall cell membrane integrity is maintained but cells loose phospholipid asymmetry. The Annexin V kits contain a membrane impermeant DNA binding dye to evaluate membrane integrity and Annexin V to assess membrane asymmetry. When cells are double stained three different populations can be observed. Healthy cells are double negative, necrotic or late apoptotic cells are double positive, and early apoptotic cells are positive for the Annexin V only.



Early Stage

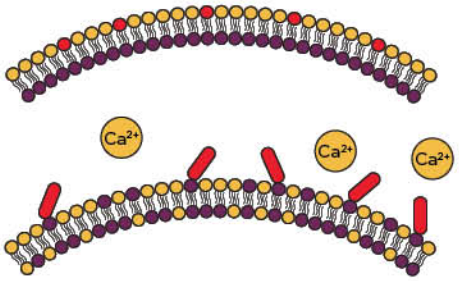
- Phosphatidyl-serine exposure
- Pore formation in mitochondria
- Activation of Caspase 8 and 9

Mid Stage

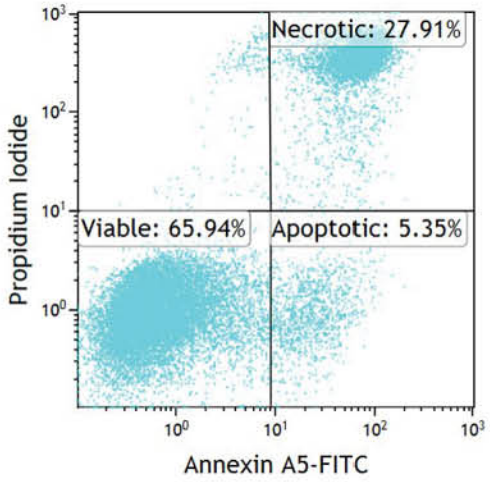
- Cell volume decreases
- Activation of Caspase 3, 6, 7
- Activated Nucleases

Late Stage

- DNA fragmentation
- Nucleus disintegrates
- Apoptotic bodies



Membrane Asymmetry Loss. Phoshpatidyl Serine residues (PS, magenta) are typically located on the inner side of the plasma membrane. During apoptosis this asymmetry is lost and PS residues flip to the outer side. In the presence of calcium ions Annexin V binds to PS residues.



Jurkat cell staining with Annexin A5-FITC Apoptosis Detection Kit.

Part Number	Size	Reagent	Excitation Max	Emission Max
IM2375	20 tests	Annexin A5-FITC	492 nm	520 nm
IM3546	200 tests	PI	550 nm	680 nm
IM3614	150 tests	Annexin A5-FITC	490 nm	520 nm
		7-AAD	543 nm	655 nm

CYTOFLEX FLOW CYTOMETER

The CytoFLEX Platform is a revolutionary system presenting optimal excitation and emission, minimizing light loss and maximizing sensitivity. Since its initial unveiling, the compact system with innovative technology borrowed from the telecommunications industry has garnered attention from the flow cytometry community. Since that time, we have continued to expand the platform, creating even more choices for researchers.

We continue to leverage the power of the platform:

- Exquisite sensitivity
- Small particle analysis in a benchtop analyzer
- Extensive set of repositionable band pass filters
- Flexibility to upgrade by adding additional parameters
- Intuitive software to facilitate multicolor analysis

beckman.com/CytoFLEX

Up to 3 lasers

Up to 4 lasers

Up to 6 lasers

Part Number	Product	Reagent	Laser	Bandpass
B88526	DNA Dye	7-AAD	488 or 561 nm	610/20
B25595	DNA Dye	DRAQ7	561 or 638 nm	690/50, 712/25, 763/43, or 780/60
B30437	DNA Dye	DAPI	355 or 375 nm	450/45
C36614	Fixable Viability Dye	ViaKrome 405	405 nm	450/45
C36620	Fixable Viability Dye	ViaKrome 561	561 nm	585/42
C36624	Fixable Viability Dye	ViaKrome 638	638 nm	660/10
C36628	Fixable Viability Dye	ViaKrome 808	808 nm	885/40
C03551	Cell Cycle Kit	PI	488 or 561 nm	585/42, 610/20, or 690/50
IM2375 & IM 3546	Apoptosis Kit	Annexin A5-FITC	488 nm	525/40
		PI	488 or 561 nm	585/42, 610/20, or 690/50
IM3514	Apoptosis Kit	Annexin A5-FITC	488 nm	525/40
		7-AAD	488 or 561 nm	610/20

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