Flow Reagents

Reducing workflow burden in high-parameter cytometry with ready-to-use reagents

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Poster #187

Invitrogen™ LIVE/DEAD™ Fixable ReadyTube and ReadyPlate viability reagents **accurately discriminate** live and dead cells across cytometry platforms while **eliminating DMSO, -20 °C storage and multiple workflow steps**.

Abstract

As flow cytometry panels continue to expand in parameter count, the demands placed on sample preparation workflows have increased substantially. Many reagents central to these workflows require 4 °C or -20 °C storage and repeated pipetting steps, introducing variability and limiting opportunities for standardization. To explore alternatives that may ease these constraints, we investigated a room-temperature–stable, ready-to-use viability reagent designed to reduce sample handling while maintaining performance comparable to existing tools. We evaluated the reagent's performance across a representative set of experimental conditions, including whole blood, cultured cells, fixed and permeabilized samples, and high-density staining formats. Live cell discrimination was assessed relative to commonly used viability reagents on both conventional and spectral flow cytometers. Across sample types and platforms, the ability to discriminate live and dead cells was within ±10% of benchmark reagents, indicating that long-term ambient storage did not diminish analytical performance. The reagent also performed consistently at high cell loads (up to 10^7 cells per tube and 10^6 per well in 96-well plates), supporting its use in high-throughput and multiparameter workflows where sample concentrations can vary substantially. A key aim of the study was to assess whether ready-to-use formats could reduce workflow complexity. Pre-dispensed tubes and 96-well plates allowed users to introduce samples directly, eliminating pipetting steps typically associated with viability staining. Stability studies at 4 °C and 23 °C showed consistent functional performance after more than 18 months of storage. These findings suggest that shelf-stable viability reagents may help streamline the increasingly complex workflows associated with expanded flow cytometry panels by reducing preparation time, minimizing sources of variability, and relaxing cold-chain requirements. Overall, this work illustrates the potential for shelf-stable, ready-to-use reagents to address key bottlenecks in modern cytometry workflows and to support continued growth in panel dimensionality without a corresponding increase in sample preparation burden.

Addressing workflow burden

Current viability reagents are supplied as lyophilized solids requiring -20 °C storage. Use requires:

- Thawing and DMSO reconstitution.
- Pipetting dye into each sample tube or plate well.
- Immediate use after reconstitution due to dye hydrolysis
- Single-use aliquots; remaining reagents are often discarded.

As panels expand, every extra pipetting and reagent-preparation step increases hands-on time, variability, and barriers to automation. ***These are bottlenecks that have been identified by the cytometry community as a key obstacle to standardization.***

Testing methods

Cell models
- Jurkat (lymphoma, small), PBMCs (mixed lymphocytes, monocytes), U937 (monocytes, large)

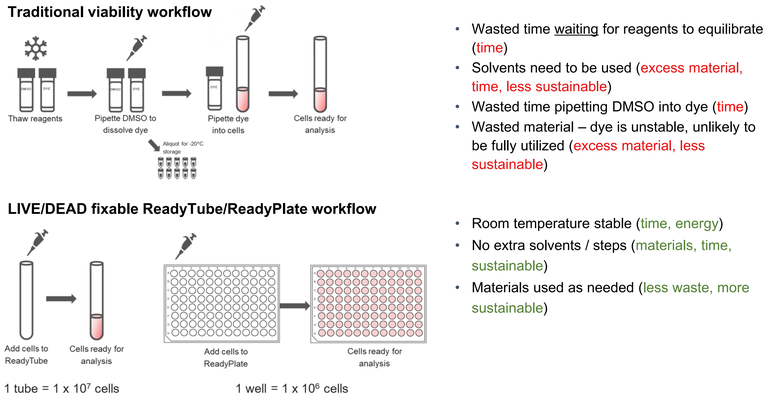
Instruments
- Invitrogen™ Attune™ CytPix™ Flow Cytometer (conventional, PMT)
- CyTek™ Aurora 5L Flow Cytometer (spectral, APD)
- Invitrogen™ Attune™ Xenith™ Flow Cytometer (spectral, PMT)

Reagents tested
- LIVE/DEAD Fixable Aqua, Blue, Near-IR and Violet viability dyes in traditional, ReadyPlate and ReadyTube formats

Test conditions
- Viability spanning 10% to 90% dead cells (heat killed)
- Fresh vs. 4% formaldehyde fixed samples
- Multicolor panel: CD3 FITC, CD4 SB780, CD8 PE-Cy7, CD19 NR700; compensation with Invitrogen™ ArC™ Amine Reactive compensation beads or stained cells
- Accelerated and real-time stability

LIVE/DEAD Ready formats simplify setup

Figure 1 | Pre-dispensed LIVE/DEAD Fixable ReadyTube and ReadyPlate formats eliminate -20 °C storage and use of DMSO while reducing workflow burden and waste.

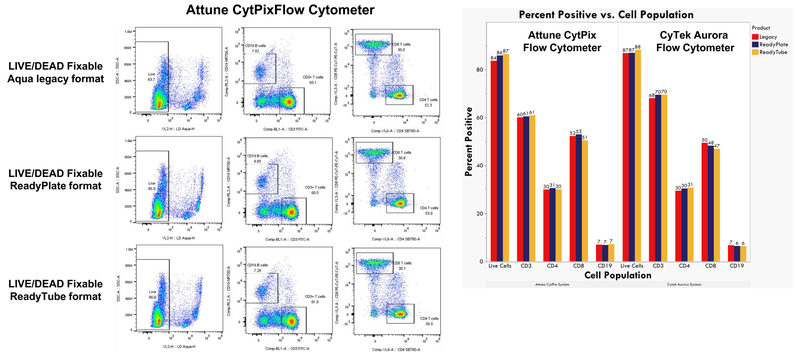


- Wasted time waiting for reagents to equilibrate (time)
- Solvents need to be used (excess material, time, less sustainable)
- Wasted time pipetting DMSO into dye (time)
- Wasted material – dye is unstable, unlikely to be fully utilized (excess material, less sustainable)

- Room temperature stable (time, energy)
- No extra solvents / steps (materials, time, sustainable)
- Materials used as needed (less waste, more sustainable)

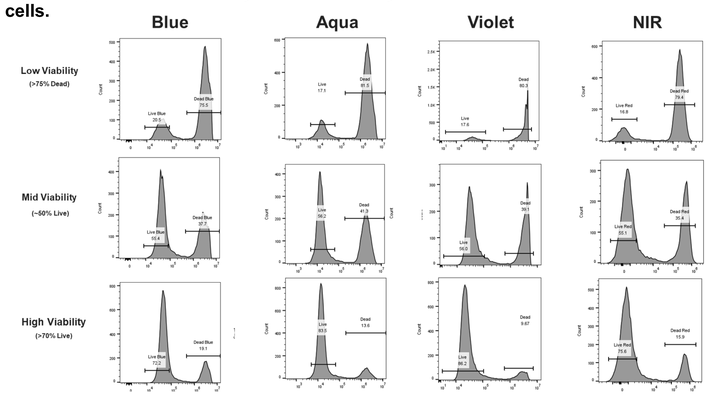
Equivalent performance to legacy formats

Figure 2 | LIVE/DEAD ReadyTube and ReadyPlate formats match legacy formats across instrument platforms.



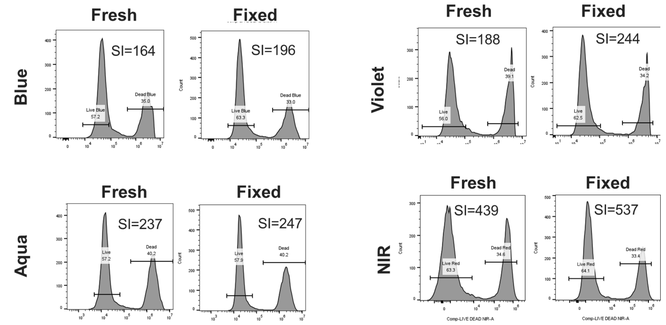
Wide dynamic range

Figure 3 | LIVE/DEAD ReadyPlate and ReadyTube formats discriminate 10% to 100% live cells.



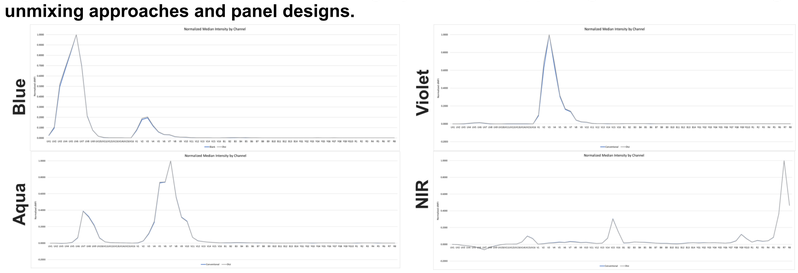
Robust to fixation

Figure 4 | Live and dead cell discrimination is preserved after 4% formaldehyde fixation.



Spectral signatures match legacy formats

Figure 5 | Spectral signatures match legacy formats, supporting transfer of existing unmixing approaches and panel designs.



Spectral Similarity Index vs. Legacy Formats

Product	Overall	UV	Violet	Blue	Yellow/Green	Red
LIVE/DEAD Aqua Ready Plates	99.982	99.984	99.996	99.962	99.833	99.982
LIVE/DEAD Blue ReadyPlates	99.993	99.993	99.994	99.946	99.853	99.983
LIVE/DEAD NIR Ready Plates	99.998	99.997	99.991	99.958	99.996	100.000
LIVE/DEAD Violet Ready Plates	99.917	99.695	99.918	99.934	99.899	99.995

High reproducibility and long-term stability

Figure 6 | Ready-to-use formats ensure reproducibility well-to-well and tube-to-tube for >18 months at 4 °C or >6 months at 23 °C.

Product	% rCV (well-well, tube-tube)	4 °C Stability	23 °C Stability
LIVE/DEAD Violet ReadyTubes	4.7%	>18 months	>6 months
LIVE/DEAD NIR ReadyTubes	4.1%		
LIVE/DEAD Aqua ReadyTubes	2.5%		
LIVE/DEAD Blue ReadyTubes	1.0%		
LIVE/DEAD NIR ReadyPlates	4.4%		
LIVE/DEAD Violet ReadyPlates	4.2%		
LIVE/DEAD Aqua ReadyPlates	3.0%		
LIVE/DEAD Blue ReadyPlates	4.5%		

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KEY PERFORMANCE METRICS

Ready-to-Use | **±10% viability of existing products** | **DMSO-free** | **Automation-friendly** | **Room-temperature-stable**

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