CTL-LDA™
Live/Dead/Apoptotic PBMC Counting

Accurate, quantitative measurement of cell viability is essential to determine the quality and functional state of peripheral blood mononuclear cells (PBMC), primarily after shipment and cryopreservation. Unimpaired samples show live cells in high frequency and few dead cells. Apoptotic cells are still alive, but will die during the course of functional assays, and therefore need to be identified as such so that the numbers of functional cells are accurately determined. Since impaired samples can show accelerated rates of apoptosis, serial measurements of apoptosis are advisable (Wunsch et al., *Cells,* 2015, 4:45). CTL has developed a three-color, fluorescence imaging-based PBMC counting platform for live, dead, and apoptotic cells: CTL-LDA™. This test platform is high-throughput suitable and enables GLP-compliant work including quality control and audit trails.

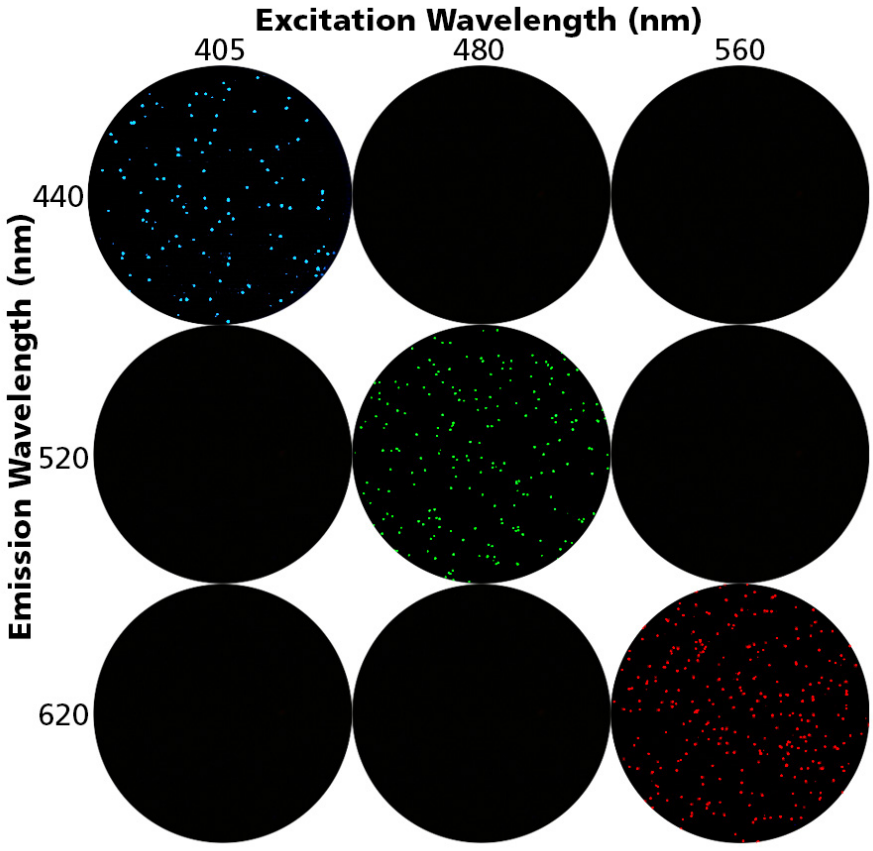
CTL-LDA™: Fluorescence-based, single-cell imaging of live/dead/and apoptotic PBMC

Live, dead, and apoptotic PBMC are identified with dyes that stain cells based on their membrane permeability. Live cells are detected at 480/520nm only, dead cells at 560/620nm only, and apoptotic cells at 405/440nm only. Therefore, no cross-bleeding of signal between the dyes is detectable *(Figure 1),* providing unambiguous identification of the state of each cell's viability.

Advantages over flow cytometric analysis

We performed viability testing with the imaging-based CTL-LDA™ Platform vs. standard flow cytometry measurements on freeze-thawed PBMC. Both methods showed similar percentages for the three states of viability as well as similar numbers of total viable cells present in the sample. However, the CTL-LDA™ Platform was far less labor intensive, provides direct imaging of cells, and eliminates

ASSAY PRINCIPLE



Stressed cells have been imaged to obtain increased numbers of dead and apoptotic cells.

CTL-LDA: Live/Dead/Apoptotic PBMC Counting

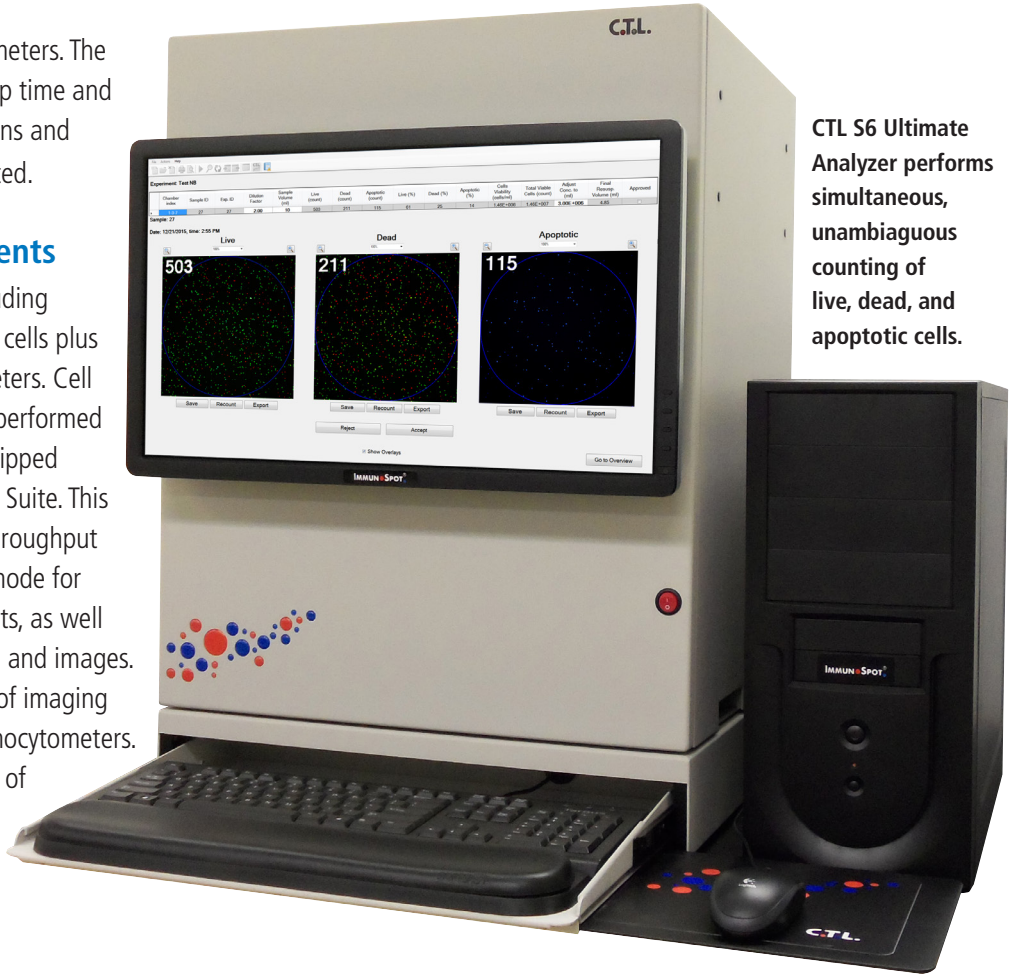
Features	CTL-LDA™ Platform	FACS Analysis	Count by Eye (Trypan Blue)
Detects live, dead, and apoptotic cells	✔	✔	✖
Direct visualization of results	✔	✖	✔
Time for scanning and data acquisition (30 samples)	~15 minutes	Hours	Hours
Simplified protocol/efficient workflow	✔	✖	✖
Automated analysis and evaluation	✔	✖	✖
Audit trails	✔	✖	✖
Assay consultation available	✔	✖	✖

Quantitation of live, dead, and apoptotic cells does not have to be complicated, ambiguous, undocumented, or time consuming. Join the community of researchers using the CTL-LDA™ Platform for unambiguously assessing the quality of cryopreserved or shipped PBMC. Contact us today for more information.

subjectivity in setting counting parameters. The CTL-LDA™ Platform requires no set up time and less maintenance. Reports, calculations and audit trails are automatically generated.

Instrumentation and Reagents

CTL provides Cell Counting Kits including reagents for the selective staining of cells plus disposable 10-chamber hemocytometers. Cell imaging, counting, and analysis are performed with a CTL S6 Ultimate Analyzer equipped with the CTL Cell Counting Software Suite. This Software allows streamlined, high-throughput cell counting with a quality control mode for additional oversight of counted results, as well as easy export of the counted results and images. The CTL S6 Analyzer is also capable of imaging individual stained cells on slides/hemocytometers. Its multi-filter design allows imaging of copious wavelengths/dyes and various cell types.



CTL S6 Ultimate Analyzer performs simultaneous, unambiaguous counting of live, dead, and apoptotic cells.

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