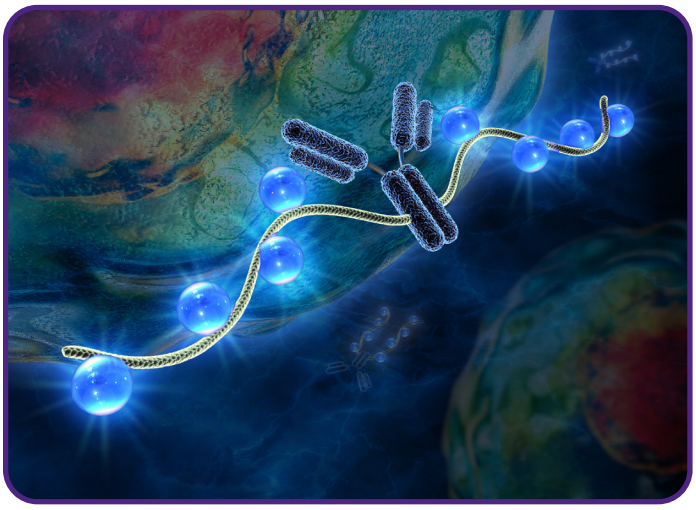
KIRAVIA Blue 520™ Antibodies

A Sparkling Advancement for Flow Cytometry



KIRAVIA Dyes™

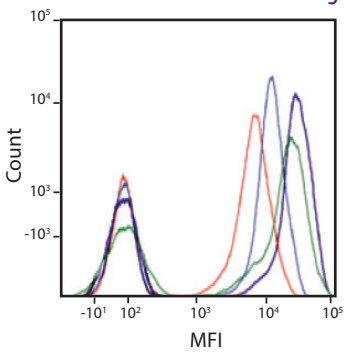
Introducing the KIRAVIA Dyes™, a brand new fluorescent chemistry to advance applications in multicolor flow cytometry and beyond. KIRAVIA is a coined term meaning "sparkling" in Japanese. KIRAVIA Blue 520™ is the first in this new family of fluorophores. KIRAVIA Dyes™ employ a unique organic backbone that separates fluorophores to minimize quenching effects, thus allowing optimal and higher fluorophore to protein (F:P) ratios, surpassing what is possible with direct conjugations of single fluorophores like FITC.

- An upgrade over FITC in terms of brightness.
- A bright and spectrally clean fluorophore that only exhibits significant spillover into Spark Blue™ 550.
- Few, if any, fluors spill into KIRAVIA Blue 520™.
- Utilize this fluor on ubiquitously expressed markers or markers with varying levels of expression.

KIRAVIA Blue 520™ conjugates are twice as bright as FITC conjugates on average, offering a brighter alternative in panel building. KIRAVIA Blue 520 ™ can be used to detect antigens with a variety of abundance levels, but would ideally be saved for an antigen with ubiquitous or variable expression levels in complex multicolor panels (*e.g.* activation markers like CD25 and CD127).

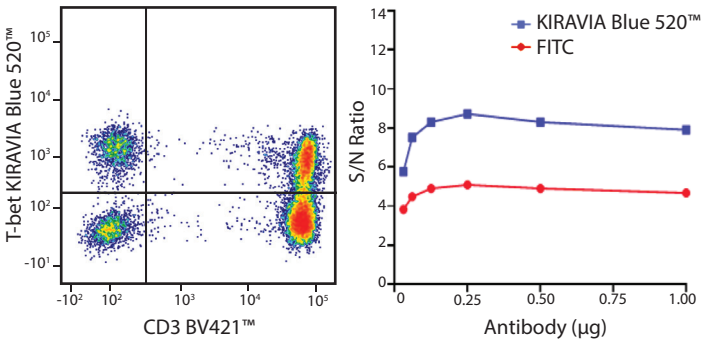
View the most up-to-date list of KIRAVIA Blue 520™ products at:
biolegend.com/en-us/kiravia

Amazing Resolution



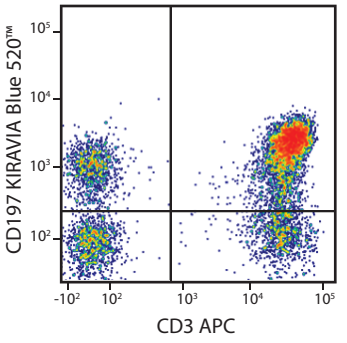
Anti-human CD4 (clone SK3), conjugated to FITC (red), Alexa Fluor® 488 (blue), BD Horizon™ Brilliant Blue 515 (green), or KIRAVIA Blue 520™ (purple) was used to stain human lysed whole blood.

Nuclear Staining and Titration



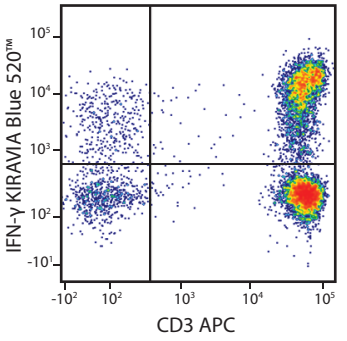
(Left) Anti-human CD3 (clone UCHT1) BV421™ and anti-human T-bet (clone 4B10) KIRAVIA Blue 520™ staining on human PBMCs. (Right) Titration curve with FITC or KIRAVIA Blue 520™ conjugated to anti-T-bet antibody.

Surface Staining



Human lysed whole blood was stained with anti-human CD3 APC (clone UCHT1) and anti-human CCR7 (clone G043H7) KIRAVIA Blue 520™.

Intracellular Staining

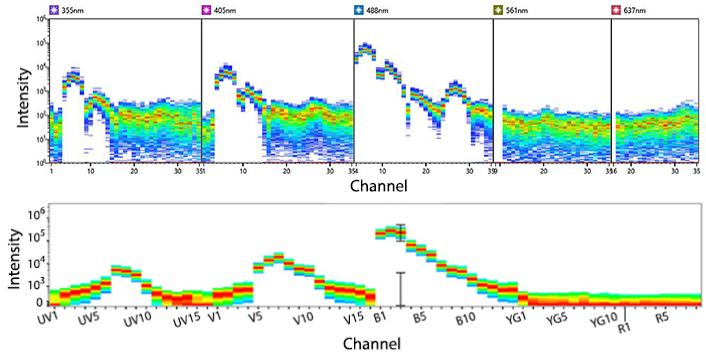


PMA/ionomycin-stimulated (6 hours) human PBMCs stained with anti-human CD3 (clone UCHT1) APC and anti-IFN-γ (clone 4S.B3) KIRAVIA Blue 520™.

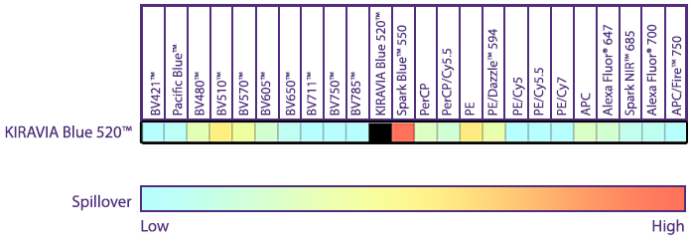
BioLegend®

KIRAVIA Blue 520™

A Brighter Alternative to FITC with Minimal Spillover

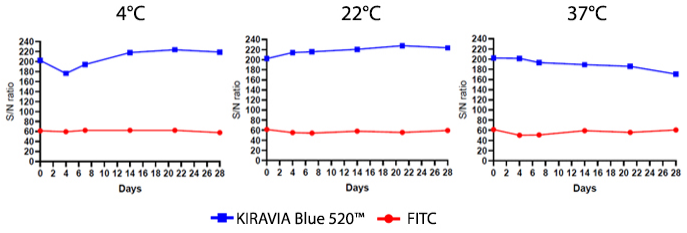


Emission spectra of KIRAVIA Blue 520™ as run on a (top) SONY ID7000™ Spectral Cell Analyzer or (bottom) 5-laser Cytek™ Aurora Spectral Cytometer.



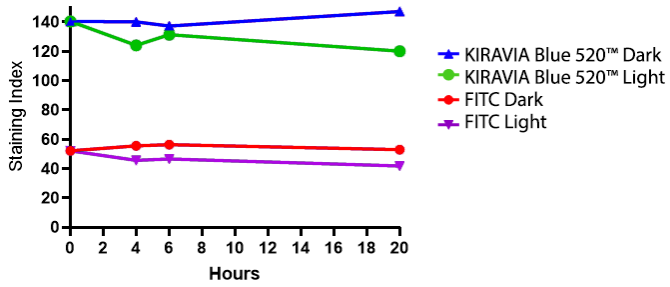
Spillover impact of KIRAVIA Blue 520™ into detection channels of a 5-laser Cytek™ Aurora Spectral Cytometer.

Heat Stability



Anti-human CD3 (clone UCHT1) antibody conjugated to KIRAVIA Blue 520™ was incubated at the indicated temperatures over the course of 28 days. The antibodies were then used to stain human lysed whole blood cells. S/N ratio was determined as ratio of the MFI$^+$/MFI$^-$ signals.

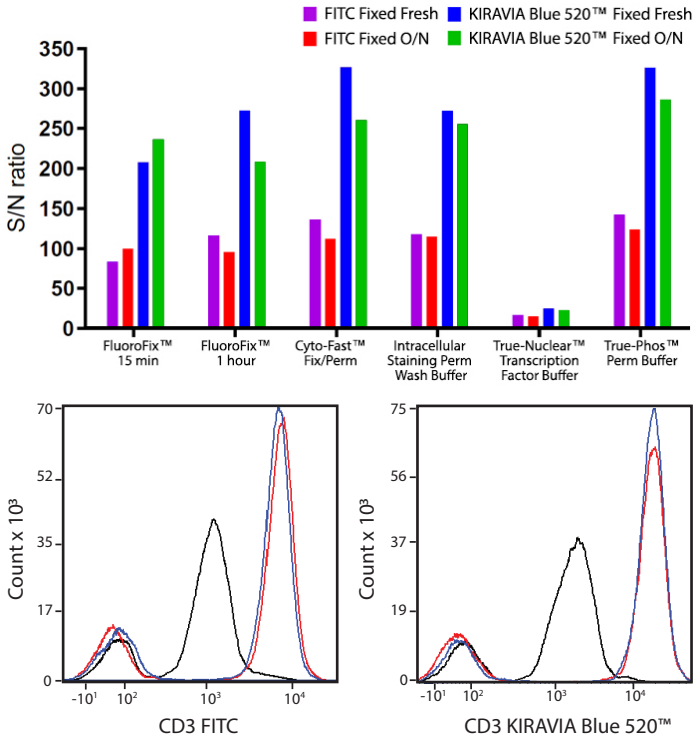
Photostability



Human PBMCs were stained with anti-human CD3 (clone UCHT1) conjugated to either FITC or KIRAVIA Blue 520™. After staining, samples were fixed and stored in FluoroFix™ Buffer through the duration of the experiment. Samples were continually exposed to light or kept in the dark and read on a BD LSR Fortessa™ instrument.

Fixative Stability

KIRAVIA Blue 520™ shows utility with most BioLegend fixation buffers. Similar to some fluorophores, KIRAVIA Blue 520™ can be sensitive to the nuclear permeabilization and fixation process if used as a surface stain conjugate. While a decrease in the MFI$^+$ signal was observed when staining with the FITC and KIRAVIA Blue 520™ conjugates, the positive population could still be resolved easily. The ability of a fluorophore conjugate to survive the fix/perm process will depend on the level of antigen expression and the sample type. For additional help, please contact our technical services group.



Human PBMCs were stained with anti-human CD3 (clone UCHT1) conjugated to either FITC or KIRAVIA Blue 520™ and then fixed as indicated. (Top) Fixed Fresh samples were fixed according to their respective protocols and read on a cytometer immediately. O/N samples were fixed by the indicated buffer and stored overnight in Cyto-Last™ Buffer before being read on the cytometer. Signal to Noise (S/N) was determined as ratio of the MFI$^+$/MFI$^-$ signals. (Bottom) Histogram overlay of the CD3 staining on PBMCs after treatment with True-Nuclear™ (black), Cyto-Fast™ (blue), or FluoroFix™ (red) as per their respective protocols.

A guide to the fixatives used in this experiment:

- FluoroFix™ is a gentler fixation PFA-based buffer.
- Cyto-Fast™ Fix/Perm Buffer Set is a specialized buffer for cytokine detection and offers improved staining over the Intracellular Staining Permeabilization Wash Buffer.
- True-Nuclear™ Transcription Factor Buffer Set is designed for optimal staining of intranuclear targets.
- True-Phos™ Perm Buffer is an alcohol-based buffer ideal for staining of phospho protein targets.
- Cyto-Last™ Buffer is formulated for long-term storage of samples while preserving signal over time.

BioLegend®