## **Technical Data Sheet**

# **BV421 Mouse Anti-Human Ki-67**

#### **Product Information**

**Material Number:** 562899

Alternate Name: MKI67; Antigen identified by monoclonal antibody Ki-67; KIA

Size Vol. per Test: 5 μl B56 Clone: Human Ki-67 Immunogen:

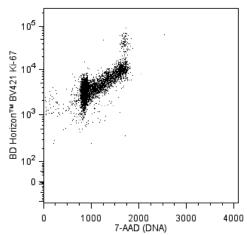
Isotype: Mouse IgG1, κ Reactivity: QC Testing: Human

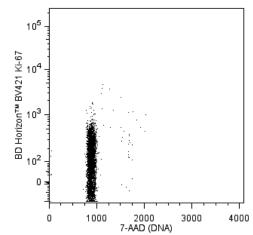
Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

## Description

The B56 monoclonal antibody specifically binds to the Ki-67 antigen that is expressed in the nucleus of cycling cells (G1, S, G2, M cell cycle phases). During the G0 phase, the antigen cannot be detected. During interphase of the cell cycle, it is associated with nucleolar components, and it is on the surface of the chromosomes during M phase. Ki-67 is a large protein having 2 alternatively spliced isoforms, an N-terminal forkhead-associated domain, a C-terminal domain that binds to heterochromatin proteins, and multiple phosphorylation sites, the functions of which are still unclear. Because of the strict association of Ki-67 expression with cell proliferation, anti-Ki-67 antibodies are useful for the identification, quantification, and monitoring of growing cell populations.

The antibody was conjugated to BD Horizon™ BV421 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. With an Ex Max of 407-nm and Em Max at 421-nm, BD Horizon™ BV421 can be excited by the violet laser and detected in the standard Pacific Blue™ filter set (eg, 450/50-nm filter). BD Horizon™ BV421 conjugates are very bright, often exhibiting a 10 fold improvement in brightness compared to Pacific Blue<sup>TM</sup> conjugates.





Multicolor flow cytometric analysis of Ki-67 expression by proliferating Molt-4 and noncycling human peripheral blood mononuclear cells (PBMC). Proliferating Molt-4 cells and noncycling PBMC were fixed and permeabilized with 70% ice cold ethanol, washed, and stained with BD Horizon™ BV421 Mouse Anti-Human Ki-67 antibody (Cat. No. 562899) according to the BD Biosciences support protocol, "Flow Cytometry Staining Protocol for Detection of Ki-67." The cells were then counterstained with BD Via-Probe™ [Cat. No. 555815; contains 7-Amino-Actinomycin D (7-AAD)] to stain DNA. Two-color flow cytometric dot plots showing the correlated expression patterns of 7-AAD staining versus Ki-67 were derived from gated events with the forward and side light-scatter characteristics of intact Molt-4 cells (Left Panel) or PBMC (Right Panel). Flow cytometry was performed using a BD LSR™ II Flow Cytometer System.

## Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with BD Horizon<sup>TM</sup> BV421 under optimum conditions, and unconjugated antibody and free BD Horizon<sup>TM</sup> BV421 were removed.

## **BD Biosciences**

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### **Application Notes**

### Application

Intracellular staining (flow cytometry)	Routinely Tested

### **Recommended Assay Procedure:**

For more information on detecting Ki-67 by flow cytometry please visit http://www.bdbiosciences.com/resources/protocols/detection ki 67.jsp

## **Suggested Companion Products**

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
555815	Cell Viability Solution	500 tests	(none)

#### **Product Notices**

- 1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use  $1 \times 10^{6}$  cells in a 100- $\mu$ l experimental sample (a test).
- 2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- An isotype control should be used at the same concentration as the antibody of interest. 3.
- 4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
- Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
- For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors
- Pacific Blue<sup>TM</sup> is a trademark of Molecular Probes, Inc., Eugene, OR.
- Brilliant Violet<sup>TM</sup> 421 is a trademark of Sirigen.

#### References

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Byeon I-JL, Li H, Song H, Gronenborn AM, Tsai M-D. Sequential phosphorylation and multisite interactions characterize specific target recognition by the FHA domain of Ki67. Nat Struct Mol Biol. 2005; 12(11):987-993. (Biology)

Ho DWY, Fan ST, To J, et al. Selective plasma filtration for treatment of fulminant hepatic failure induced by D-galactosamine in a pig model. Gut. 2002; 50:869-876. (Clone-specific)

Kill IR. Localisation of the Ki-67 antigen within the nucleolus: evidence for a fibrillarin-deficient region of the dense fibrillar component. J Cell Sci. 1996; 109(6):1253-1263. (Biology)

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