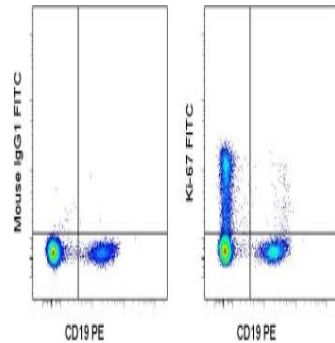


## Ki-67 FITC

**Catalog Number(s):** 9011-5699-025 (25 tests), 9011-5699-120 (120 tests)



Fluorescence profiles of normal human peripheral blood cells stimulated for 2-days with immobilized Anti-Human CD3. Cells were stained with CD19 PE followed by intracellular staining with Mouse IgG1 FITC (left) or Ki-67 FITC (right).

### Product Information



**Contents:** Ki-67 FITC

**Catalog Number(s):** 9011-5699-025 (25 tests),  
9011-5699-120 (120 tests)

**Clone:** 20Raj1

**Concentration:** 5  $\mu$ L (0.06  $\mu$ g)/test (a test is defined as the amount that will stain  
1 x 10<sup>6</sup> cells in 100  $\mu$ L)

**Host/Isotype:** Mouse IgG1

**Formulation:** Aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer.



**Storage Conditions:** Store at 2-8°C.

Do not freeze.



Light-sensitive material.



Caution, contains Azide



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### Intended Use

The 20Raj1 fluorochrome-conjugated monoclonal antibody reacts with the human Ki-67 antigen. Ki-67 can be detected in human biological samples using immunological techniques.

### Principles of the Test

Flow cytometry is a useful tool for simultaneously measuring multiple physical properties of individual particles (such as cells). Cells pass single-file through a laser beam. As each cell passes through the laser beam, the cytometer records how the cell or particle scatters incident laser light and emits fluorescence. Using this flow cytometric analysis protocol, one can

perform a simultaneous analysis of surface molecules at the single-cell level.

### Description

The monoclonal antibody 20Raj1 recognizes the human Ki-67 protein. Two isoforms of Ki-67 exist, a 345 and 395 kDa form that are expressed in dividing cells. Ki-67 is expressed in all cell types and is detectable during active phases of the cell cycle (G1, S, G2, and mitosis) but is absent from resting cells (G0). During interphase, Ki-67 expression is localized to the nucleus but redistributes to the chromosomes during mitosis and has specifically been found to associate with heterochromatin-bound proteins such as chromobox protein homolog 3 (CBX3).

### Specimen Collection and Storage Instructions

Collect venous blood sample by venipuncture into a sterile blood collection tube using an appropriate anticoagulant (EDTA is recommended). Keep samples at room temperature (18-25°C). Prior to use, mix samples by gentle agitation.

### Materials Required But Not Provided

- 12x75 mm test tubes
- Buffers (eBioscience Flow Cytometry Staining Buffer, Cat. No. 00-4222 recommended)
- Lysis Buffer (eBioscience 1X RBC Lysis Buffer, Cat. No. 00-4333 or eBioscience 1-step Fix/Lyse Solution (10X), Cat. No. 00-5333 recommended)
- For intracellular staining use IC Fixation Buffer and Permeabilization Buffer, Cat. No. 88-8823 (intracellular cytokine or cytoplasmic protein staining) or Foxp3 Buffer Set, Cat. No. 00-5523 (For nuclear protein staining). Refer to the Best Protocols section of the eBioscience website for the "Staining Intracellular Antigens for Flow Cytometry" protocols.
- Viability stain (7-AAD Viability Staining Solution, Cat. No. 00-6993 or Propidium Iodide Staining Solution, Cat. No. 00-6990 recommended)
- Automated pipettes
- Centrifuge
- Vortex mixer
- Ice bucket or refrigerator
- Flow cytometer

### Test Protocol

NOTE: For intracellular staining, refer to the Best Protocols section of the eBioscience website for the "Staining Intracellular Antigens for Flow Cytometry" protocols.

1. Aliquot 100 µL of the test sample into tubes.
2. Add 5 µL of the appropriate antibody to each tube.
3. Incubate 30-60 minutes at 2-8°C. Alternatively, samples can be incubated at room temperature in the dark 15-30 minutes.
4. Add 2 ml of 1X RBC Lysis Buffer (at room temperature) per tube. Mix gently. (Alternatively, samples can be incubated with 2 mL 1-step Fix/Lyse Solution.)
5. Incubate samples in the dark at room temperature for 10 minutes. Do not exceed 15 minutes of incubation with the RBC Lysis Buffer.

6. Centrifuge samples at 300-400 x g for 5 minutes at room temperature, decant/aspirate supernatant and wash 1 time with 2 ml of Flow Cytometry Staining Buffer.
7. Centrifuge samples at 300-400 x g for 5 minutes at room temperature, decant/aspirate supernatant.
8. Resuspend stained cell pellet in 1 mL Flow Cytometry Staining Buffer and analyze samples on a flow cytometer.

### Limitations

1. For optimal performance of fluorochrome conjugated antibodies, store vials at 2-8°C in the dark. Do not freeze.
2. Centrifuge the antibody vial prior to opening to recover the maximum volume.
3. Except where noted in the protocol, all staining should be done on ice or at 2-8°C with minimal exposure to light.

### Performance Characteristics

Consistency of high-quality reagents is ensured by testing each lot of monoclonal antibody for conformance against characteristics of a standard reagent. Representative flow cytometric data is included where appropriate.

### Evidence of Deterioration

For questions or concerns regarding the performance or quality of products received, please contact eBioscience Technical Support (see below).

### References

Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture (H3-A6), 3rd Edition published by the National Committee for Clinical Laboratory Standards.

Schlüter C, Duchrow M, Wohlenberg C, Becker MH, Key G, Flad HD, Gerdes J. The cell proliferation-associated antigen of antibody Ki-67: a very large, ubiquitous nuclear protein with numerous repeated elements, representing a new kind of cell cycle-maintaining proteins. *J Cell Biol.* 1993 Nov;123(3):513-22

Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol.* 1984 Oct;133(4):1710-5.