

## Datasheet: BUF09C

**BATCH NUMBER 166707**

|                      |                   |
|----------------------|-------------------|
| <b>Description:</b>  | LEUCOPERM         |
| <b>Name:</b>         | LEUCOPERM         |
| <b>Format:</b>       | Reagent           |
| <b>Product Type:</b> | Accessory Reagent |
| <b>Quantity:</b>     | 1000 TESTS        |

### Product Details

#### Applications

This product has been reported to work in the following applications. This information is derived from testing within our laboratories, peer-reviewed publications or personal communications from the originators. Please refer to references indicated for further information. For general protocol recommendations, please visit [www.bio-rad-antibodies.com/protocols](http://www.bio-rad-antibodies.com/protocols).

|                | Yes | No | Not Determined | Suggested Dilution |
|----------------|-----|----|----------------|--------------------|
| Flow Cytometry | ▪   |    |                |                    |

LEUCOPERM reagents are intended for fixing cells in suspension with Reagent A and then permeabilizing the cells with Reagent B. The specific formulations reduce background staining and allow simultaneous addition of permeabilization medium and fluorochrome labeled antibodies.

#### Product Form

Reagent A - Fixation medium  
Reagent B - Permeabilisation medium

#### Preservative Stabilisers

Formaldehyde in Reagent A

#### Product Information

Flow cytometric analyses with monoclonal antibodies have been restricted primarily to cell surface molecules. Intracellular structures such as cytoplasmic or nuclear enzymes, oncoproteins, cytokines, immunoglobulins etc. were largely excluded from such assays.

Also excluded from flow cytometric assays were cytoplasmic localizations of well established membrane molecules such as CD3 and CD22.

LEUCOPERM reagents allow intracellular antigen analysis with the same ease as surface antigens. The only prerequisite is the availability of suitable antibody conjugates. Most commercially available monoclonal antibody conjugates can be used with LEUCOPERM reagents. Some determinants are sensitive, however, to the fixation step involved. This and the optimal fixation time may have to be determined experimentally for each antibody conjugate.

**Instructions For Use** For the detection of cell cycle antigens such as Ki-67, PCNA and BrdU, methanol modification is recommended - see [protocol #F5](#).

1. Prepare cells in the appropriate manner. Adjust cell suspension to a concentration of  $1 \times 10^7$  cells/ml in PBS/BSA. Whole blood samples may also be used. Bio-Rad recommend the use of EDTA anti-coagulant in these circumstances, although satisfactory results may be obtained using heparin or acid-citrate dextrose.
2. Add 100ul of cell suspension to the appropriate number of test tubes.  
If required, perform staining of cell surface antigens at this stage. Following staining for the recommended period, wash cells once in PBS/BSA and discard supernatant.
3. Perform fixation of cells using appropriate fixation medium.
4. Add 3ml PBS/BSA and centrifuge for 5 minutes at 300 x g. Remove supernatant.
5. Re-suspend cells in 100ul of BUF09CB (Permeabilization Reagent).
6. Immediately add recommended volume of the appropriate directly conjugated antibody. Vortex and incubate for 30 minutes at room temperature.  
If using an unconjugated primary antibody, wash in 3ml of PBS/BSA (as per step 5) and then repeat step 7 using an appropriate secondary antibody. There is no requirement to add further Leucoperm.
7. Incubate for 30 minutes at room temperature.
8. Wash cells with 3ml phosphate buffered saline and centrifuge for 5 minutes at 300 x g.
9. Wash once in PBS/BSA. Remove supernatant and re-suspend cells in sheath fluid for immediate analysis or re-suspend cells in 0.25ml of 0.5% formaldehyde and store them at 2-8°C in the dark. Analyse fixed cells within 24 hours.

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**Storage** LEUCOPERM Cell Permeabilisation reagents should be stored and used at room temperature. DO NOT FREEZE. Do not use reagents if a precipitate forms or discolouration occurs.

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**Guarantee** 12 months from date of despatch

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**Health And Safety Information** Material Safety Datasheet documentation #10187 #10509 available at: <https://www.bio-rad-antibodies.com/SDS/BUF09C>  
Reagent A - Fixation medium (10187)  
Reagent B - Permeabilisation medium (10509)

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