Datasheet: BUF09C

BATCH NUMBER 157139

Description:	LEUCOPERM
Name:	LEUCOPERM
Format:	Reagent
Product Type:	Accessory Reagent
Quantity:	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.

	Yes	No	Not Determined	Suggested Dilution
Flow Cytometry	▪			

LEUCOPERM reagents are intended for fixing cells in suspension with Reagent A and then permeabilising the cells with Reagent B. The specific formulations reduce background staining and allow simultaneous addition of permeabilisation medium and fluorochrome labelled 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 x 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. Add 100ul of Reagent A (fixation medium, stored at room temperature).

4. Incubate for 15 minutes at room temperature.

5. Add 3ml PBS/BSA and centrifuge for 5 minutes at 300 x g. Remove supernatant.

6. Resuspend cells in 100ul of Reagent B (Permeabilization Medium).

7. 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.

8. Wash once in PBS/BSA. Remove supernatant and resuspend cells in sheath fluid for immediate analysis or resuspend cells in 0.25ml of 0.5% formaldehyde and store them at 2-8^{o}C in the dark. Analyse fixed cells within 24 hours.

References

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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.
Guarantee	12 months from date of despatch
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)
Regulatory	For research purposes only

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