

## Datasheet: BUF09

<b>Description:</b>	LEUCOPERM™
<b>Name:</b>	LEUCOPERM™
<b>Format:</b>	Reagent
<b>Product Type:</b>	Accessory Reagent
<b>Quantity:</b>	50 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.

	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. This procedure gives antibodies access to intracellular structures and leaves the morphological scatter characteristics of the cells intact. 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

### Specificity

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 studies.

Also excluded from flow cytometric studies were cytoplasmic localisations 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 #17](#).**

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. AbD Serotec 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°C in the dark. Analyse fixed cells within 24 hours.

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#### References

1. Chiu, W.C. *et al.* (2009) Effects of dietary fish oil supplementation on cellular adhesion molecule expression and tissue myeloperoxidase activity in hypercholesterolemic mice with sepsis. [J Nutr Biochem. 20: 254-60.](#)
2. Grundy, M. *et al.* (2010) The FLT3 internal tandem duplication mutation is a secondary target of the aurora B kinase inhibitor AZD1152-HQPA in acute myelogenous leukemia cells. [Mol Cancer Ther. 9: 661-72.](#)
3. Taylor, L. *et al.* (2010) The effect of acute hypoxia on heat shock protein 72 expression and oxidative stress *in vivo*. [Eur J Appl Physiol. Mar 13. \[Epub ahead of print\]](#)
4. Myles, A. *et al.* (2011) Expression of Toll-like receptors 2 and 4 is increased in peripheral blood and synovial fluid monocytes of patients with enthesitis-related arthritis subtype of juvenile idiopathic arthritis. [Rheumatology \(Oxford\). 50: 481-8.](#)
5. Osorio, Y. *et al.* (2011) Identification of small molecule lead compounds for visceral leishmaniasis using a novel ex vivo splenic explant model system [PLoS Negl Trop Dis. 5:e962.](#)

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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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#### Shelf Life

12 months from date of despatch.

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#### Acknowledgements

Manufactured by AN DER GRUB Bio Research GmbH for MorphoSys UK Limited

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#### Health And Safety Information

Material Safety Datasheet Documentation #10187 available at: <http://www.abdserotec.com/uploads/MSDS/10187.pdf>

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**For research purposes only, unless otherwise specified in writing by AbD Serotec.**

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