

PRODUCT ANALYSIS SHEET

Catalog Number:	FB001
Lot Number:	See product label
Volume:	100 mL
Composition:	Paraformaldehyde in phosphate buffered saline, pH 7.3 (Caution: paraformaldehyde is a hazardous and poisonous substance. Handle with care and dispose of properly.)
Applications:	Fixation of cells for use in flow cytometry particularly for Intracellular staining procedures (see below for recommended protocol).
Stability:	Refer to bottle for expiration date.
Storage:	Store at 2-8°C. DO NOT FREEZE.
Intracellular Staining Protocol:	<p>Isolation and stimulation of PBMCs: PBMCs are isolated from whole blood by Ficoll-Paque density gradient separation. The cells are then stimulated [i.e. 6 hours with PMA (50 ng/mL) and calcium ionophore A23187 (250 ng/mL)] in the presence of 2 μM monensin. The cells are washed with PBS/0.1% sodium azide/1% FCS and stained with appropriate cell surface staining markers (i.e. mouse anti-human CD4 FITC conjugate cat. # MHCD0401). After washing a further 2 times, the cells are resuspended in 1 mL IC Fixation Buffer per 1×10^6 cells and incubated at 4°C for 10 minutes. To remove the paraformaldehyde the cells are washed thoroughly with PBS/0.1% sodium azide/1% FCS. The fixed cells can be stored at 4°C for up to 7 days prior to intracellular staining.</p> <p>Intracellular staining: Fixed PMBCs are aliquoted to a density of 10^6 cells/tube and washed 2 times in 1 mL IC Permeabilization Buffer. The cells are spun at 300 x g for 5 minutes, the supernatant aspirated and the cells are resuspended in 40 μL of IC Permeabilization Buffer. Add anti-cytokine conjugate (typically 0.5-1 μg) and incubate at 4°C for 30 minutes. The cells are spun at 300 x g for 5 minutes and washed 3 times in 1 mL IC Permeabilization Buffer. The cells are finally resuspended in 0.5 mL 50 mM phosphate buffered saline, pH 7.3, for flow cytometric analysis. At least one of the following specificity controls is recommended 1) appropriate isotype control (i.e. mouse IgG1 conjugate); 2) pre-incubating conjugated antibody with recombinant cytokine; 3) pre-blocking cells with unconjugated anti-cytokine antibody prior to staining with conjugated antibody.</p>
Reference:	Palmer, D.R. and U. Krzych (2002) Cellular and molecular requirements for the recall of IL-4-producing memory CD4(+)CD45RO(+)CD27(-) T cells during protection induced by attenuated <i>Plasmodium falciparum</i> sporozoites. Eur. J. Immunol. 32(3):652-661.

This product is for research use only. Not for use in diagnostic procedures.

www.invitrogen.com

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com