Intracellular Staining Kit

PRODUCT ANALYSIS SHEET

Catalog Number: ANN0001

Lot Number: See product label

IC Fixation Buffer

Catalog Number:	FB001C
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 kit label for expiration date.
Storage:	Store at 2-8°C. DO NOT FREEZE.

IC Permeabilization Buffer

Catalog Number:	PB001C
Lot Number:	See product label
Volume:	2 x 125 mL of a 5x concentrate solution. Dilute in distilled H_2O, sufficient to make 1 liter of IC Permeabilization Buffer.
Composition:	Phosphate buffered saline, pH 7.3, containing fetal calf serum, saponin and 0.1% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly).
Applications:	Permeabilization of cells for use in flow cytometry particularly for Intracellular staining procedures (see below for recommended protocol).
Stability:	Refer to kit label for expiration date.
Storage:	Store at 2-8°C. DO NOT FREEZE.

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Intracellular Staining Protocol:

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 further 2 times, the cells are resuspended in 1 mL IC Fixation Buffer per 1 x 10^6 and incubated at 4°C for 10 minutes. To remove the para-formaldehyde 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.

Intracellular staining: Fixed PBMCs 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 IgG_1 conjugate); 2) pre-incubating conjugated antibody with recombinant cytokine; 3) pre-blocking cells with unconjugated anti-cytokine antibody prior to staining with conjugated antibody.

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 *Plasmodium falciparum* sporozoites. Eur. J. Immunol. 32 (3):652-661.

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