

PRODUCT DESCRIPTION

This kit contains four conjugated antibodies (and corresponding isotype controls) that can be used for single-step staining of human Th2 cells (1 - 5):

MATERIALS PROVIDED & STORAGE

Store the unopened kit at 2-8 °C in the dark. Refer to kit label for date of expiration.

PART	PART #	DESCRIPTION
Positive Markers	967108	250 µL of IL-4 R-PE, Mouse IgG _{2A} ; Clone 25463
	967109	250 µL of STAT6-APC, Mouse IgG _{2A} ; Clone 253906
	967110	250 µL of IL-4-CFS Mouse IgG ₁ ; Clone 3007
	967094	250 µL of CD3-PerCP Mouse IgG ₁ ; Clone UCHT1
Isotype Controls	965658	250 µL of Mouse IgG _{2A} -PE Isotype Control
	965667	250 µL of Mouse IgG _{2A} -APC Isotype Control
	965668	250 µL of Mouse IgG ₁ -CFS Isotype Control
	965669	250 µL of Mouse IgG ₁ -PerCP Isotype Control
Fixation/Permeabilization Buffer	895029	30 mL of 1X Fixation/Permeabilization Buffer
Permeabilization/Wash Buffer	895030	2 bottles (30 mL/bottle) of 1X Permeabilization/Wash Buffer

INTENDED USE

This product is designed for the flow cytometric analysis of human Th2 cells using four fluorochrome-conjugated antibodies.

PRECAUTIONS

The fixation/Permeabilization Buffer provided in this kit contains Formaldehyde which is a suspected carcinogen. Avoid contact with skin, eyes, and mucous membranes, and avoid inhaling fumes. In case of contact, wash immediately with water and seek medical advice.

Some components in this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

INTRACELLULAR STAINING PROTOCOL WITH SIMULTANEOUS FIXATION/PERMEABILIZATION

1. Harvest cells of interest and wash twice in PBS or Hanks' Balanced Salt Solution (HBSS).
2. Approximately 5 x 10⁵ washed cells should be resuspended in 0.5 mL of Fixation/Permeabilization Buffer and incubated at 2-8 °C for 30 minutes. Cells should be vortexed intermittently in order to maintain a single cell suspension.
3. The cells are centrifuged and the pellet is resuspended in 100 - 200 µL of the Permeabilization/Wash Buffer.
4. Add 10 µL of each antibody or each corresponding isotype control antibody to the cells.
5. Incubate the mixture for 30 - 45 minutes at 2-8 °C in the dark.
6. Following the incubation, remove any excess antibody by washing the cells in 2 mL of Permeabilization/Wash Buffer. The final cell pellet is resuspended in 200 - 400 µL of PBS for flow cytometric analysis.

Notes: Because saponin-mediated cell permeabilization is a reversible process, it is important to keep the cells in the presence of saponin during intracellular staining. Using multiple fluorochromes requires proper flow cytometric compensation to remove the spillover fluorescence from a particular probe to a certain channel (6).

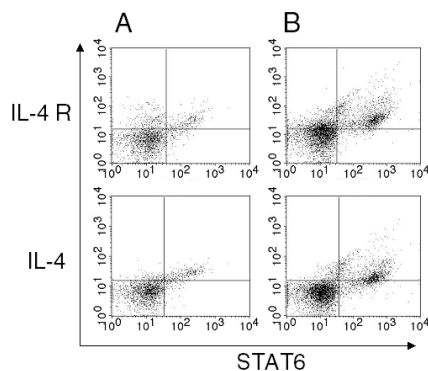


Figure 1: For Th2 activation, human PBMCs were cultured for 72 hours with recombinant human IL-4 (5 ng/mL; R&D Systems®, Catalog # 204-IL) and anti-human IFN- γ (10 μ g/mL; R&D Systems®, Catalog # AB-285-NA) followed by a 3 hour re-stimulation with PMA/ionomycin. Cells were harvested and stained with the indicated antibodies following the procedure. Dot plots show the relative IL-4 R⁺, IL-4⁺, and STAT6⁺ populations from inactivated (A) and activated (B) CD3⁺-gated PBMCs. Quadrants were set based on isotype controls.

REFERENCES

1. Chang, S. and T.M. Aune (2007) *Nature Immunol.* **8**:723.
2. Lee, G.R. *et al.* (2006) *Immunity* **24**:369.
3. Flavell, R.A. *et al.* (2002) *Inflamm. Res.* **51**:80.
4. Lee, D.U. and A. Rao (2004) *Proc. Natl. Acad. Sci. USA* **101**:16010.
5. Spilianakis, C.G. and R.A. Flavell (2004) *Nat. Immunol.* **5**:1017.
6. Bagwell, B. and E.G. Adams (1993) *Ann. N.Y. Acad. Sci.* **677**:167.