**ABSTRACT**

Although IL-17 is made by CD4+ Th17 cells and there is an ever-increasing body of literature on these cells, other cells make IL-17, including CD8+ T cells, NK cells, and neutrophils. There are many factors that contribute to published findings of the percentage of cells producing IL-17, including the activation or differentiation time course of activation, and antibody used for detection. It has been published that high levels of IL-17 are secreted by human PBMCs after anti-CD3/CD28-treatment alone for only 24 hrs (Chen, Z. et al. (2007) J. Exp. Med. 200, 3719-3732). For research use only. Not for use in diagnostic procedures.

**MATERIALS AND METHODS**

**CELLS AND ACTIVATION**

Primary human CD4+ T cells were purified from peripheral blood leukocytes. In some experiments, PBMCs were stimulated with 50 ng/mL PMA and 200 ng/mL ionomycin in the presence of 3% milk for 3 days. In other experiments, 500 ng/mL PMA and 10 ng/mL rhIL-23 (R&D Systems Catalog # 1290-IL) was also used. PBMCs were stained with diI-AAC or diO-AAC to distinguish resting and activated cells. Cells were harvested on day 3 and IL-17 protein was detected by flow cytometry and Western blot. The ELISA protocol was used to confirm the antibody specificity.

**DEVELOPMENT & TESTING OF MONOCLONAL ANTI-HUMAN IL-17A ANTIBODY (CLONE 41802)**

- **Western blot with rhIL-17A**
- **Direct ELISA with rhIL-17A**
- **Intracellular flow cytometry of HEK293 cells overexpressing rhIL-17A**
- **Intracellular flow cytometry of resting and activated PBMCs**
- **Western blot and Western blot (Catalog # MAB3171, IC3171P, IC3171C).**

**ABBRs were transfected with SEIP vector alone, or with human IL-17A or IL-17F. Cells were harvested and stained with anti-human IL-17A (Catalog #409515, 1:1000; mouse), surface control (Catalog # RB10008) followed by PE-conjugated mouse secondary antibody (Catalog # FC010).**

**DETECTION OF HUMAN IL-17A IN HEK293-IL-17A TRANSFECTANTS**

**WESSTERN BLOT DETECTION OF HUMAN IL-17A IN TH17 CELLS**

Primary human CD4+ T cells were purified from peripheral blood leukocytes via negative selection (R&D Systems Catalog # 7350-07). Cells were differentiated into Th17 cells as previously described (J Exp Med 2007 200, 3719-3732). For research use only. Not for use in diagnostic procedures.