

# PERFORMANCE DATA SHEET

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## Monoclonal anti-human FOXP3/FITC\*

**mAb name/Clone:** ANCFX2D7

**Isotype:** Mouse IgG1k

**Immunogen:** Recombinant human FOXP3

**CATALOG#:** 333-040

**QUANTITY:** 120 tests

**VOLUME IN VIAL:** 0.2ml

**WORKING DILUTION:** 1:50 (or use 1.6µl of concentrated stock per  $5 \times 10^5$ -cell test)

**INFORMATION:** The FOXP3 molecule is a 50–55 kD transcription factor also known as IPEX, JM2, Forkhead box 3, and Scurfin. Defects in this gene can result in lethal autoimmune disease (2). When used with cell surface markers CD4 and CD25(IL-2R), FOXP3 is a useful marker for identifying T regulatory cells. Functionally, FOXP3 is thought to mediate oxidative phosphorylation, enabling Treg to function in a lower oxygen environment (3).

In EIA, clone ANCFX2D7 binds to full length recombinant FOXP3 and to a FOXP3(R1-R3) construct (aa 1–198) but not to a FOXP3(R1) construct (aa 1-70), suggesting that its epitope is within regions 2 and 3 (aa 71–198).

ANCFX2D7 binds to nuclear FOXP3 found constitutively in a 0.5 – 3% sub population of fixed, permeabilized CD4+ peripheral blood lymphoid cells in FACS

**FOXP3 References:** 1) G Roncador, A H Banham, et al. (2005) *Eur J Immunol* **35**(6): 1681-1691. PMID: 15902688. 2) S Hori, S Sakaguchi, (2004) *Microbes and Infection* **6**(8): 745-751. 3) U H Beier, W W Hancock, et al. (2016) *J Immunol* **196**(1 suppl 211.3).

**STORAGE CONDITIONS:** Store at 2 - 5°C. Freeze/Thawing is not recommended. **Protect from light.**

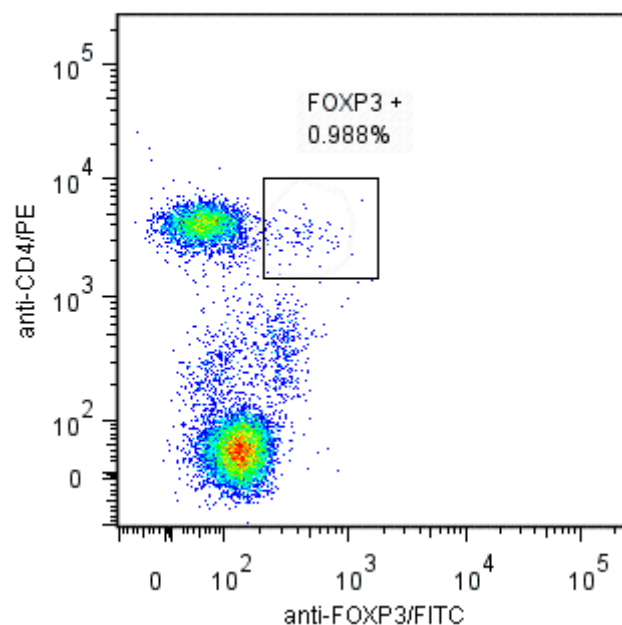
**PRODUCT STABILITY:** Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date: \_\_\_\_\_

**BUFFER:** 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 5% Glycerol, 0.2% BSA, 0.04% NaN<sub>3</sub> (as a preservative).

**PRODUCTION:** Protein A purified antibody from tissue culture supernatant was reacted with FITC. Unconjugated FITC was separated from antibody/FITC conjugate by desalting column. The antibody/FITC conjugate is at **0.1 mg/ml** with a Fluorescein/IgG molar ratio of 11.6.

**PERFORMANCE:** Five  $\times 10^5$  ficoll prepared human peripheral blood mononuclear cells (PBMC) per tube were washed and pre stained with anti-CD4/R-PE (Catalog #148-050). They were washed twice and fixed with NFP (Nuclear Fix/perm) buffer for 30 minutes, after which they were washed three times in NPR (Nuclear Permeabilization) buffer. Subsequent incubations and washes were done using this buffer. Fixed cells were then pre incubated 10 minutes with 20 µl of 200 µg/ml Mouse IgG (to reduce non specific binding) after which they were incubated 30 minutes on ice with 80 µl of anti-FOXP3/FITC at a **1:50** dilution factor (2 µg/ml). They were then washed three times and fixed with 2% Formaldehyde/PBS and analyzed by FACS using a lymphoid gate. A net **1.0 % sub population** of the cells stained positive with a mean shift of **0.65 log<sub>10</sub>** fluorescent units when compared to a Mouse IgG1/FITC negative control (Catalog #278-040). Binding was blocked when cells were pre incubated 10 minutes with unlabeled anti-FOXP3 antibody (Catalog #333-020).

**Binding of anti-FOXP3/FITC to human PBL**



**\*Research use only. Not for use in Diagnostic procedures.**

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