PERFORMANCE DATA SHEET
2441

Monoclonal anti-human FOXP3/Biotin *

mAb name/Clone: **ANCFX2D7**
Isotype: Mouse IgG1k
Immunogen: Recombinant human FOXP3

CATALOG#: 333-030
QUANTITY: 100 µg **CONCENTRATION: 1.0 mg/ml**

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, permeabolized 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^{o}C***. Freeze/Thawing is not recommended.

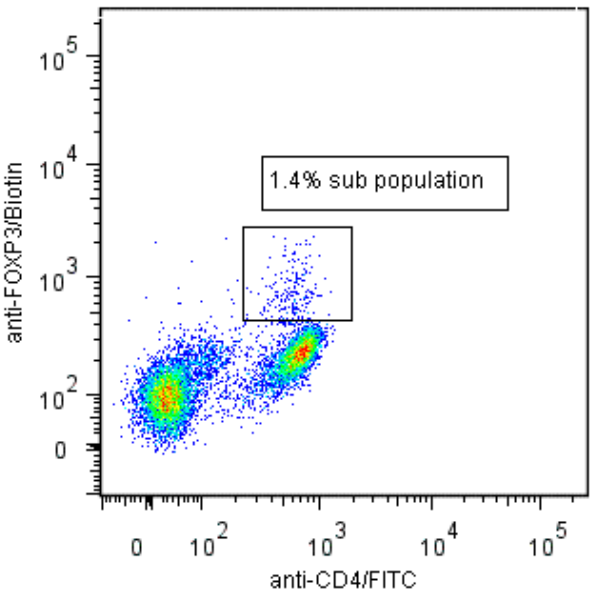
PRODUCT STABILITY: Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date:_____

BUFFER: 50 mM Sodium Phosphate pH 7.5, 100 mM Potassium Chloride, 150mM NaCl, 5% Glycerol, 0.2% BSA, 0.04% NaN_3 (as a preservative).

PRODUCTION: Antibody from (low FBS containing) tissue culture supernatant was Protein A purified to >95% mouse immunoglobulin by SDS-PAGE (<1% bovine immunoglobulin), and reacted with NHS-Biotin. Unconjugated Biotin was removed from conjugate using a desalting column.

PERFORMANCE: Five x 10^5 ficoll prepared human peripheral blood mononuclear cells (PBMC) per tube were washed and pre stained with anti-CD4/FITC (Catalog #148-040). 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 Permeabolization) buffer. Subsequent incubations and washes were done using this buffer. Fixed cells were then pre incubated 10 minutes with 20 ul of 200 ug/ml Mouse IgG (to reduce non specific binding) after which they were incubated 30 minutes on ice with 80 ul of anti-FOXP3/Biotin at a concentration of 2 ug/ml. Cells were washed twice and incubated 30 minutes on ice with 2^{o} detection reagent Streptaviden/PE (Catalog # 253-050). They were then washed three times and fixed with 2% Formaldehyde/PBS and analyzed by FACS using a lymphoid gate. A net **1.4% sub population** of the cells stained positive with a mean shift of **0.71** $\log_{10}$ fluorescent units when compared to a Mouse IgG1/Biotin negative control (Catalog #278-030). Binding was blocked when cells were pre incubated 10 minutes with unlabeled anti-FOXP3 antibody (Catalog #333-020).

Binding of anti-FOXP3/Biotin +SA/PE to human PBMC



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