PERFORMANCE DATA SHEET
3032

Monoclonal anti-human FOXP3/R-PE *

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

CATALOG#: 333-050
QUANTITY: 120 tests **VOLUME IN VIAL: 0.2ml**
WORKING DILUTION: 1:50 (use 1.6µl per 5 x 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, 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.*** **Do not freeze! Protect from light.**

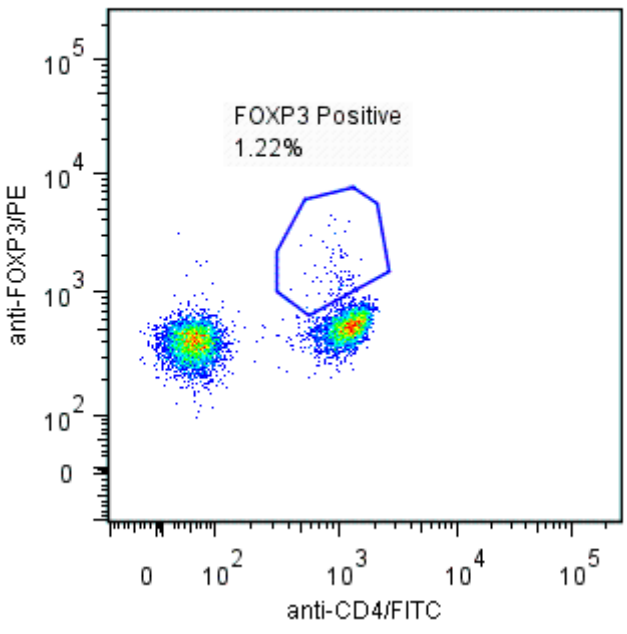
PRODUCT STABILITY: Product should retain activity for at least 12 months after shipping date when stored as recommended. Ship Date:_____

BUFFER: 50 mM Sodium Phosphate pH 7.5, 500 mM Potassium Chloride, 150mM NaCl, 15% Glycerol, 0.2% BSA, 0.04% NaN_3 (as a preservative).

PRODUCTION: Protein A purified antibody from tissue culture supernatant was conjugated to R-Phycoerythrin through a sulfo-ester linkage. Unconjugated antibody was removed using size exclusion chromatography. Antibody conjugate is at **125 µg/ml** with an A_{565}/A_{280} ratio of 3.68.

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 Nuclear Fix/perm buffer for 30 minutes, after which they were washed three times in Nuclear Permeabolization buffer. Subsequent incubations and washes were done using this buffer. Fixed cells were then pre incubated 10 minutes with 20 ul of 500 ug/ml Mouse IgG (to reduce non specific binding) and 15% Mouse sera, after which they were incubated 30 minutes on ice with 80 ul of anti-FOXP3/PE at a **1:50 dilution** (2.5 ug/ml). Cells were three times, fixed with 2% Formaldehyde/PBS and analyzed by FACS using a lymphoid gate. A **1.2% sub population** of the cells stained positive with a mean shift of **1.02** $\log_{10}$ fluorescent units when compared to a Mouse IgG1/PE negative control (Catalog #278-050). Binding was blocked when cells were pre incubated 10 minutes with an excess of unlabeled anti-FOXP3 antibody (Catalog #333-020).

Binding of anti-FOXP3/PE to CD4+ human PBL



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