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# New Item New Item

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## Transcription factor selective antibodies

### Forkhead box protein 3 (Fox3p) Antibodies (Cat # Fox3P-101AP, P-Fox3P and PC-Fox3P)

Alternate nomenclature: Scurfin, forkhead box 3, zinc finger protein JM2,

Forkhead box protein P3 (Fox3p) is a probable transcription factor that plays a critical role in the control of immune response. Mice deficient in FOX3P (Scurfy mice) exhibited over proliferation of CD4+CD8- T lymphocytes, multi-organ infiltration and elevation of numerous cytokines. The Fox3P deficiency is lethal in hemizygous mice similar to CTLA4 or TGFbeta deficient mice, the pathology observed in scurfy mice is similar to animals that lack expression of CTLA4 or TGF beta that result from inability to properly regulate CD4+CD8- T cell activity (1). strong correlation between expression of forkhead box P3 and ex vivo expression of CTLA-4 in Treg cells was observed. The protein encoded by scurfin gene, designated as FOX3P is a new member of the forkhead/winged-helix family of transcriptional regulators and is highly conserved in humans and is essential for normal immune homeostasis. FoxP3 is a necessary transcription factor for the development and function of CD4+ regulatory T-cells (Tregs) and it inhibits HIV infection by inhibiting NFAT activity and NFAT proteins contribute to HIV-1 transcription (2). Mutations in FOX3P gene (homolog of mouse scurfy gene) resulted in IPEX (X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome) disorder characterized by immune disorder characterized by immune dys-regulation, insulin dependent diabetes mellitus, infection, secretory diarrhea, thrombocytopenia, anemia and eczema. It is usually lethal in infancy. The CD4+CD25+ exert their suppressor function by regulating miR-142-3p via Adenylyl cyclase 9 (AC9) that inhibits AC9 production in Tregs. The FOX3P down-regulates miR-142-3p to keep the AC9-cAMP pathway active in Cd4+CD25+ Treg (3). The forkhead box p3 (Foxp3) positive Tregs have functionally immunosuppressive properties that prevents effector cells from acting against self in autoimmune diseases/tumors are highly relevant in cancer progression (4).

Fox3P is a soluble nuclear protein containing 431 amino acids (MW 52kDa) protein contains 1 C2H2-type zing finger and one forkhead DNA-binding domain. Fox3P is expressed in several splice variant forms. Epigenetic regulation of Fox3P locus contributes to its role as a lineage-specification factor for development of Tregs. The Fox3PP gene is located on chromosome Xp11.23-Xq13.3. Forkhead (FH), also known as a 'winged helix', is named for the Drosophila fork head protein, a transcription factor which promotes terminal rather than segmental development. Disruption in Fox3pp/winged helix/scurfy protein led lethal lymphoproliferative disorder in scurfin mouse. The scurfin has several FH (forkhead DNA binding domains near C-terminal end of the protein. At least 2 variant isoforms are expressed, a longer iso-form a and a short iso-form b.

The Fox3pp-selective antibodies were generated against synthetic peptide form unique region near the C-terminal end of mouse Fox3pp protein, this peptide sequence is not present in other proteins from the gene bank. FabGennix Inc. has generated rabbit anti-Fox3pp mono-epitope-specific antibodies utilizing linear and cyclic peptide methodology. The Anti-Fox3pp antibodies have been fully characterized for cross reactivity with other members of the scurfin protein in cells form rat, mouse and human origin using Western blot analyses. The Fox3pp antibody is available as FITC-conjugate, other conjugates for this antibody can be ordered at a nominal charge. FabGennix International Inc., has also produced antibodies to several other transcription factors. For a complete listing please visit [www.FabGennix.com](http://www.FabGennix.com). FabGennix antibodies are also available worldwide from our distribution centers. FabGennix Int. Inc., also provide western blot positive controls for Fox3pp in ready-to-use buffer. Limited quantities of the antigenic blocking peptide for Fox3pp antibodies is also available (inquire for availability).

Catalog #	Host Species	Nature	Cross reactivity	Quantity	volume
Fox3-101AP	Rabbit	Affinity purified Fox3 antibodies	M, H, bovine, monkey	100ug	200ul
P-Fox3	Rabbit	Antigenic blocking peptide for Fox3-101AP	R, M, H, monk	100ug	100ul
PC-Fox3	Rabbit	Western blot positive control for Fox3	R, M, H, monk	250ug	inquire

R = rat; M = mouse; H = human; C = chicken; monk = monkey ; \* not all variants are labeled equally

**Immunogen:** Synthetic peptides are selected from forkhead/winged helix transcription factor 3 (Fox3p) corresponding to amino acids 229-250 of mouse protein (amino acids aqc llq rev vqs leq qle leke), the peptide was post-synthetically modified to achieve desired antigenicity before coupling to a carrier protein.

**Concentration:** Fox3-101AP IgG concentration 0.65-1.25 mg/ml.

**Applications:** Antibody Fox3-101AP is ideal for IMM and WB. Fox3-101AP has not been tested in other applications. The dilutions for these antibodies are for reference only, investigators are expected to determine the optimal conditions for specific assay. WB: 1:500; IMM & i.p pull-down assays:> 1:200 (2-5ul/250 ug protein extracts).

**Reactivity:** This antibody detects a single 52-55kDa Fox3p protein in PC-Fox3p samples. The antibody also reacts to a smaller (25-29kD) protein in many cells lines, the identity of this protein is not known.

**Protocols:** Standard protocol for various applications (WB, IMM and IHC) of this antibody is provided with the product specification sheet, however, FabGennix Inc., suggest that end-users standardize the conditions for specific applications in the lab.

**Form/Storage:** The antiserum is supplied in antibody stabilization buffer with 0.02% sodium azide. For long-term storage of antibodies, store at -20°C. FabGennix Int. Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody dilution buffer (Cat # DiUbuffer). Working solutions of antibodies in DiUbuffer should be filtered through 0.45µm filter after every use for long-term storage.

**Notes:** Briefly centrifuge to collect liquid, heat or boil PC-TLR6 tube for 1-2 minutes to dissolve any precipitate before use. This product is "ready-to-use" for electrophoresis. After thawing store at room temperature, Repeated freezing and thawing may result in appearance of higher MW immunoreactive bands.

**New Reagents:** Now you can recycle your western blots (nitrocellulose, supported membranes and PVDF membranes) by using our StripObuffer (Cat FGI-1989). Each stripping is guaranteed to give better signal (up to 8 stripping). No strong pungent smell of reducing agents or heating required. Block in 5 x diluObuffer and you are ready for blotting with a new antibody

#### References:

1. Brunkow ME, Jeffery EW, Hjerrild KA, Paepfer B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F. Disruption of a new forkhead/winged-helix protein, scurfy, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001 Jan;27(1):68-73.
2. Selliah N, Zhang M, White S, Zoltick P, Sawaya BE, Finkel TH, Cron RQ. FOXP3 inhibits HIV-1 infection of CD4 T-cells via inhibition of LTR transcriptional activity. Virology. 2008 Nov 25;381(2):161-7. Epub 2008 Oct 1
3. Huang B, Zhao J, Lei Z, Shen S, Li D, Shen GX, Zhang GM, Feng ZH. miR-142-3p restricts cAMP production in CD4(+)CD25(-) T cells and CD4(+)CD25(+) T(Reg) cells by targeting AC9 mRNA. EMBO Rep. 2008 Dec 19. [Epub ahead of print].
4. Jang TJ. Prevalence of Foxp3 Positive T Regulatory Cells is Increased during Progression of Cutaneous Squamous Tumors. Yonsei Med J. 2008 Dec 31;49(6):942-8.

\*For users who may require large amounts of Fox3-101AP, please enquire about bulk material discounts.  
This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

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