

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse FoxO3 in Western blots.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human FoxO3 Met372-Gly673 Accession # O43524
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

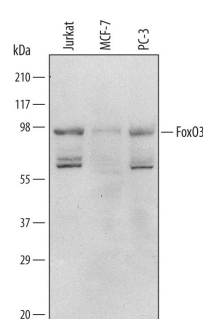
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the Technical Information section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Simple Western	10 µg/mL	MCF-7 human breast cancer cell line
Knockout Validated	FoxO3 is specifically detected in HEK293T human embryonic kidney parental cell line but is not detectable in FoxO3 knockout HEK293T cell line.	

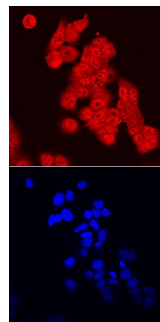
DATA

Western Blot



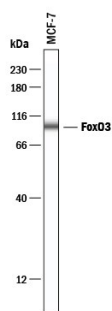
Detection of Human FoxO3 by Western Blot. Western blot shows lysates of Jurkat human acute T cell leukemia cell line, MCF-7 human breast cancer cell line, and PC-3 human prostate cancer cell line. PVDF Membrane was probed with 1 µg/mL of Sheep Anti-Human FoxO3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6165) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for FoxO3 at approximately 90 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



FoxO3 in MCF-7 Human Cell Line. FoxO3 was detected in immersion fixed MCF-7 human breast cancer cell line using Sheep Anti-Human FoxO3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6165) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red, upper panel; Catalog # NL010) and counterstained with DAPI (blue, lower panel). Specific staining was localized to nuclei and cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

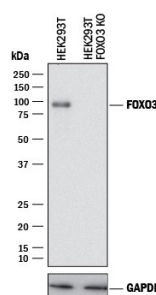
Simple Western



Detection of Human FoxO3 by Simple Western™. Simple Western lane view shows lysates of MCF-7 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for FoxO3 at approximately 97 kDa (as indicated) using 10 µg/mL of Sheep Anti-Human/Mouse FoxO3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6165) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.



Knockout Validated



Western Blot Shows Human FoxO3 Specificity by Using Knockout Cell Line. Western blot shows lysates of HEK293T human embryonic kidney parental cell line and FoxO3 knockout HEK293T cell line (KO). PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human/Mouse FoxO3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6165) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for FoxO3 at approximately 90 kDa (as indicated) in the parental HEK293T cell line, but is not detectable in knockout HEK293T cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution Reconstitute at 0.2 mg/mL in sterile PBS.

Shipping The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C

Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Forkhead box O3 (FoxO3) is a ubiquitously expressed 72 kDa transcriptional regulator that is involved in cellular differentiation, angiogenesis, tumor progression, apoptosis, and the responses to oxidative stress and DNA damage. Phosphorylation of FoxO3 by Akt induces its association with 14-3-3 proteins and its retention in the cytoplasm. In response to the loss of survival factors, dephosphorylation of FoxO3 induces its translocation to the nucleus where it promotes apoptosis. Its level of acetylation is regulated in response to cellular metabolic requirements. Within amino acids 372-673 (C-terminal to the DNA binding domain), human FoxO3 shares 95% aa sequence identity with mouse and rat FoxO3.