

Phospho-FOXO4 (Thr28) Recombinant Polyclonal Antibody (12HCLC)

Product Details	
Size	100 µg
Species Reactivity	Human
Host/Isotype	Rabbit / IgG
Expression system	Expi293
Class	Recombinant Polyclonal
Type	Antibody
Clone	12HCLC
Conjugate	Unconjugated
Immunogen	phosphopeptide corresponding to human FOXO4 (aa 25-33)
Form	Liquid
Concentration	0.5 mg/mL
Purification	Protein A
Storage buffer	PBS, pH 7.2
Contains	0.09% sodium azide
Storage conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.
RRID	AB_2532776

Applications	Tested Dilution	Publications
Western Blot (WB)	1-3 µg/mL	-
Immunohistochemistry (Paraffin) (IHC (P))	1:20	-
Immunocytochemistry (ICC/IF)	1 µg/mL	-

Product Specific Information

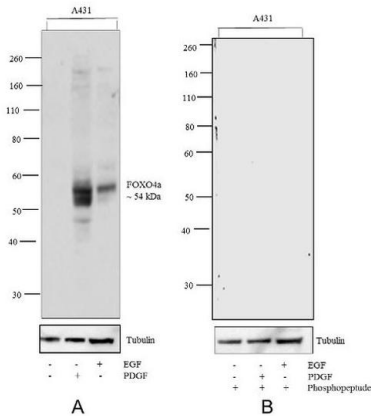
This antibody is predicted to react with Monkey, Goat, Pig, Bovine, Dog, Rat and Mouse.

Recombinant rabbit polyclonal antibodies are unique offerings from Thermo Fisher Scientific. They are comprised of a selection of multiple different recombinant monoclonal antibodies, providing the best of both worlds - the sensitivity of polyclonal antibodies with the specificity of monoclonal antibodies - all delivered with the consistency only found in a recombinant antibody. While functionally the same as a polyclonal antibody - recognizing multiple epitope sites on the target and producing higher detection sensitivity for low abundance targets - a recombinant rabbit polyclonal antibody has a known mixture of light and heavy chains. The exact population can be produced in every lot, circumventing the biological variability typically associated with polyclonal antibody production.

Product Images For Phospho-FOXO4 (Thr28) Recombinant Polyclonal Antibody (12HCLC)

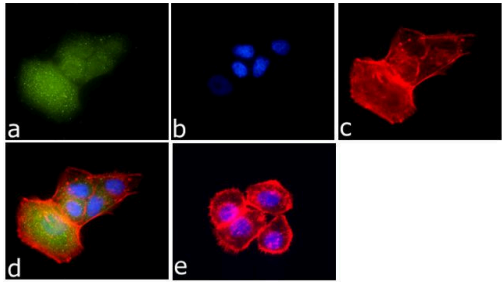
Phospho-FOXO4 (Thr28) Antibody (710880) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of A431 (Lane 1), A431 treated with PDGF (150 nM/10 min) (Lane 2), A431 treated with EGF (200 ng/mL for 10 min) (Lane 3). The blots were probed with Anti-FoxO4 (pT28) Recombinant Rabbit Polyclonal Antibody (Product # 710880, 1-3 µg/mL) and detected by chemiluminescence using Goat anti-Rabbit IgG (Heavy Chain) Superclonal Secondary Antibody, HRP conjugate (Product # A27036, 0.4 µg/mL, 1:2500 dilution). A clear 55 kDa band corresponding to FoxO4 (pT28) was observed across cell lines tested. B) To confirm the specificity of Anti-FoxO4 (pT28) Recombinant Rabbit Polyclonal Antibody, competition was performed with the phosphopeptide (10 µg/mL) as shown in the corresponding blot on right. The peptide competes with the antibody and prevents it from binding to the membrane. Known quantity of protein samples were electrophoresed using Novex®NuPAGE®4-12% Bis-Tris gel (Product # NP0321BOX), XCell SureLock Electrophoresis System (Product # EI0002), and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane by overnight wet transfer method. The membrane was probed with the relevant primary and secondary antibody following blocking with 5% skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western blotting Substrate (Product # 32106).



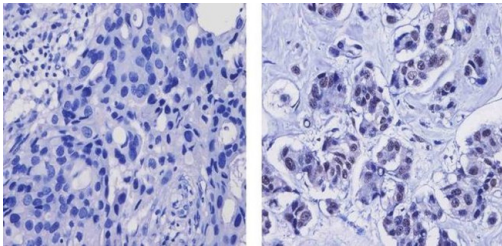
Phospho-FOXO4 (Thr28) Antibody (710880) in ICC/IF

Immunofluorescence was performed on fixed and permeabilized A431 cells for detection of FoxO4 (pT28) using Anti-FoxO4 (pT28) Recombinant Rabbit Polyclonal Antibody (Product # 710880, 1 µg/mL) and labeled with Goat anti-Rabbit IgG (Heavy Chain) Superclonal Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034, 0.4 µg/mL, 1:2500). Panel a) shows representative cells that were stained for detection and localization of FoxO4 (pT28) (green), Panel b) is stained for nuclei (blue) using SlowFade® Gold Antifade Mountant with DAPI (Product # S36938, 1:50). Panel c) represents cytoskeletal F-actin staining using Alexa Fluor® 594 Phalloidin (Product # A12381, 1:200). Panel d) is a composite image of Panels a, b and c clearly demonstrating cytoplasmic and nuclear localization of FoxO4 (pT28). Panel e) represents control cells with no primary antibody to assess background.



Phospho-FOXO4 (Thr28) Antibody (710880) in IHC (P)

Immunohistochemistry analysis of Foxo4 showing staining in the nucleus of paraffin-embedded human breast carcinoma (right) compared to a negative control without primary antibody (left). To expose target proteins, antigen retrieval was performed using 10mM sodium citrate (pH 6.0), microwaved for 8-15 min. Following antigen retrieval, tissues were blocked in 3% H2O2-methanol for 15 min at room temperature, washed with ddH2O and PBS, and then probed with a Foxo4 Recombinant Rabbit Polyclonal Antibody (Product # 710880) diluted in 3% BSA-PBS at a dilution of 1:20 for 1 hour at 37°C in a humidified chamber. Tissues were washed extensively in PBST and detection was performed using an HRP-conjugated secondary antibody followed by colorimetric detection using a DAB kit. Tissues were counterstained with hematoxylin and dehydrated with ethanol and xylene to prep for mounting.



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