# Rabbit (polyclonal) Anti-FOXO3 [pS$^{207}$]/FOXO1 [pS$^{212}$] Phosphospecific Antibody, Unconjugated

## PRODUCT ANALYSIS SHEET

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|---|---|
| **Catalog Number:** | 44-1230G (10 mini-blot size) |
| **Lot Number:** | See product label |
| **Volume:** | 100 µL |
| **Form of Antibody:** | Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without $Mg^{2+}$ and $Ca^{2+}$), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier. |
| **Preservative:** | 0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.) |
| **Purification:** | Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively pre-adsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated FOXO3. The final product is generated by affinity chromatography using a FOXO3-derived peptide that is phosphorylated at serine 207. |
| **Immunogen:** | The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human FOXO3 that contains serine 207. |
| **Target Summary:** | Forkhead-like protein -1 (FKHR-L1, also known as FOXO3) is a ~ 78 kDa member of a family of at least 43 transcriptional regulators that are involved in differentiation, transformation and metabolism. Deregulation of these proteins leads to congenital disorders, diabetes mellitus and carcinogenesis. FOXO3 is phosphorylated on several serine and threonine sites by several upstream kinases including Akt, SGK1 and MST1. Oxidative stress induces MST1-mediated phosphorylation of FOXO3 at serine 207 leading to the dissociation of FOXO3 from 14-3-3 proteins and its accumulation in the nucleus, which in turn, induce the expression of cell death genes. Phosphorylation of FOXO3 at serine 207 by MST1 mediates its role in cellular homeostasis and longevity. |
| **Reactivity:** | Human FOXO3. Mouse FOXO3 (100% homology) is expected to react. This antibody is expected to detect FOXO1 at serine 212 (100% homology). |
| **Applications:** | The antibody has been used for Western blotting applications. |
| **Suggested Working Dilutions:** | For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. The optimal antibody concentration should be determined empirically for each specific application. |
| **Storage:** | Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient. |
| **Expiration Date:** | Expires one year from date of receipt when stored as instructed. |
| **Positive Controls Used:** | Hek293 cells co-expressing wild type FOXO3 and active MST1. |

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**Related Products:**

**Antibodies:**

FOXO1 [pT$^{24}$]/FOXO3 [pT$^{32}$], Cat. # 44-1240G

Akt [pS$^{473}$], Cat. # 44-622G

Akt [pT$^{308}$], Cat. # 44-602G

PRAS-40 [pT$^{246}$], Cat. # 44-1100G

mTOR [pS$^{2448}$], Cat. # 44-1125G

AMPKα [pT$^{172}$], Cat. # 44-1150G

GS muscle [pSpS$^{641/645}$], Cat. # 44-1092G

GSK-3β [pS$^{9}$], Cat. # 44-1600G

PTEN [pS$^{370}$], Cat. # 44-1060G

PTEN [pSpTpS$^{380/382/385}$], Cat. #44-1066G

PTEN [pS$^{385}$], Cat. #44-1064G

**References:**


Lehtinen, M.K., et al. (2006) A conserved MST-FOXO signaling pathway mediates oxidative-stress responses and extends life span. Cell 125 (5):987-1001. (*This phosphorylation site specific antibody is cited in this paper.)*

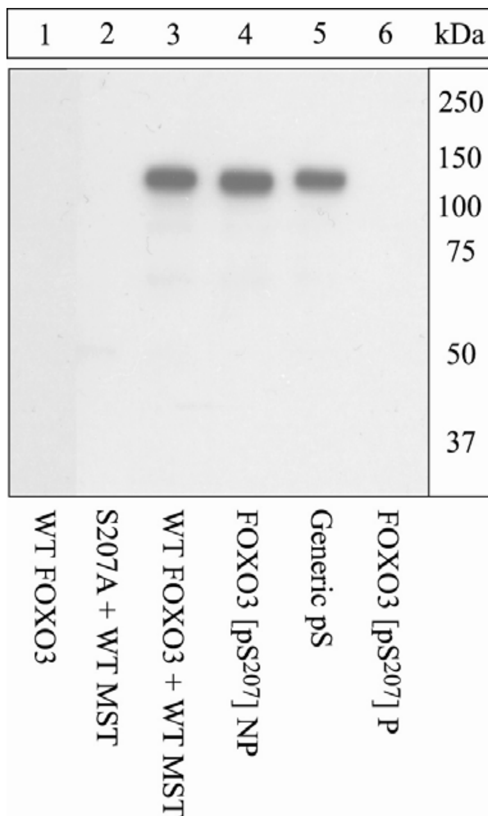
Greer, E.L. and A. Brunet (2005) FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene 24(50):7410-7425. Review.

Brunet, A., et al. (2004) Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 303(5666):2011-2015.

Buergering, B.M. and G.P. Coffer (2002) Cell cycle and death control: long live Forkheads. Trends Biochem. Sci. 27(7):352-360. Review.

Dijkers, P.F., et al. (2000) Forkhead transcription factor FKHR-L1 modulates cytokine-dependent transcriptional regulation of p27 (KIP1). Mol. Cell Biol. 20(24):9138-9148.

Tran, H., et al. (2002) DNA repair pathway stimulated by the forkhead transcription factor FOXO3a through the Gadd45 protein. Science 296(5567):530-534.


**Antibody Specificity**

Lysates prepared from Hek293 cells transfected with wild type GFP-FOXO3 alone (1) mutant S207A GFP-FOXO3 co-transfected with wild type MST1 (2), or wild-type FOXO3 co-transfected with wild-type active MST1 (3-6), were resolved on a 10% polyacrylamide gel and transferred to PVDF. The membrane was blocked with a 3% BSA-TBST buffer for one hour at room temperature, and then incubated with the FOXO3 [pS$^{207}$] antibody for two hours at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (3), the non-phosphopeptide corresponding to the phosphopeptide immunogen (4), a generic phosphoserine containing peptide (5), or the phosphopeptide immunogen (6). After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method. The data show that MST1 induced the phosphorylation of FOXO3 at serine 207 and that the signal was abolished in cells over-expressing mutant S207A FOXO3 or in the presence of inactive MST1 kinase. The data also show that the signal is blocked only by the phosphopeptide corresponding to FOXO3 [pS$^{207}$], indicating again its specificity.

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**Western Blotting Procedure**

1. Lyse approximately $10^7$ cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100$^o$C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with dd$H_2O$ for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4$^o$C.
12. Incubate the blocked blot with primary antibody at a 1:1000 dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4$^o$C or for one hour at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat F(ab')$_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

**Cell Lysis Buffer Formulation:**
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM $Na_3VO_4$
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

**Transfer Buffer Formulation:**
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4$^o$C prior to use.

**Tris Buffered Saline Formulation:**
20 mM Tris-HCl, pH 7.4
0.9% NaCl

**Blocking Buffer Formulation:**
100 mL Tris buffered saline
3 gm Ig-free BSA
0.1 mL Tween 20

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