FOXO3a pSer7 (mouse; residues 3-11), pAb

Alternate Names: Foxo3, Forkhead in Rhabdosarcoma-Like 1; FKHRL1

Cat. No.	**68-0037-100**	Quantity:	100 µg
Lot. No.	**30276**	Storage:	-20˚C

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CERTIFICATE OF ANALYSIS | **Page 1 of 2**

This antibody was developed and validated by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (University of Dundee, Dundee, UK).

Background

Protein ubiquitylation and protein phosphorylation are two major post-translational modifications that regulate the functions of proteins in eukaryotic cells. However, these modifications do not operate independently of one another, but are frequently interlinked to enable biological processes to be controlled in a more complex and sophisticated manner. Studying how protein phosphorylation events control the ubiquitin system and how ubiquitylation regulates protein phosphorylation has become a focal point of the study of cell regulation and human disease. Cloning of Forkhead Box O3a (FOXO3a) was first described by Anderson *et al.* (1998). FOXO3a belongs to the forkhead family of transcription factors, it is a member of the O subclass which can be characterized by a distinct fork head DNA-binding domain. Three other FOXO family members exist in humans, FOXO1, FOXO4 and FOXO6. These transcription factors share the ability to be inhibited and translocated out of the nucleus upon phosphorylation by enzymes such as Akt/PKB from the PI3K signaling pathway (Brunet *et al.*, 1999; Skurk *et al.*, 2005). FOXO3a has been shown to upregulate pro-apoptotic genes, such as Bim and PUMA, or downregulate anti-apoptotic genes such as FLIP (Ekoff *et al.*, 2014; Skurk *et al.*, 2005). De-

Continued on page 2

Physical Characteristics

Quantity: 100 µg

Concentration: to be provided on shipping

Source: sheep polyclonal antibody

Immunogen: mouse FOXO3a (residues 3 – 11) [EAPA(pS)PVPL]

Purification: affinity-purified against phosphopeptide

Formulation: phosphate-buffered saline

Specificity: detects FOXO3a at ~82-97 kDa

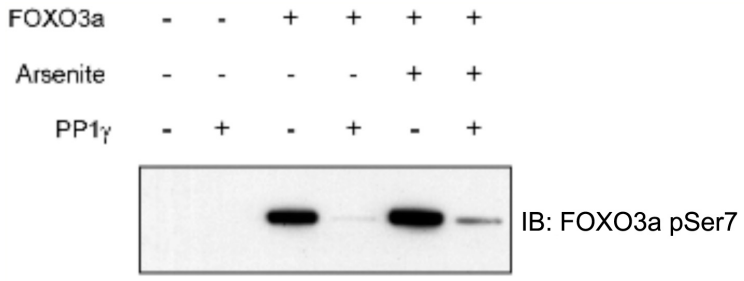
Reactivity: mouse; other species not tested

Stability/Storage: 12 months at -20˚C; aliquot as required

Research Applications and Quality Assurance

Western Immunoblotting:
use 1 µg/ml; add 10 µg of the non-phosphorylated form of the peptide immunogen (Cat# 68-1000-001 provided) to your immunoblotting incubation per 1 µg of polyclonal antibody in order to deplete any non-phospho specific polyclonal antibodies present.

Immunoprecipitation:
not tested



Western Blotting Analysis:
FLAG-FOXO3a was transfected into HEK293 cells and the cells were serum starved overnight and then stimulated with or without arsenite (0.5 M) for 60 mins. Immunoprecipitation was subsequently performed from 100 µg cell lysate using a commercially available anti-FLAG antibody. The immunoprecipitate was treated +/- 1 mU of PP1γ for 30 mins at 30°C. FOXO3a pSer7 was subsequently detected by Western Blot using the anti-FOXO3a pSer7 antibody (Cat# 68-0037-100).

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Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

FOXO3a pSer7 (mouse; residues 3-11), pAb

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Background

Continued from page 1

regulation of FOX family transcription factors (including FOXO3a) has a crucial role in the development and progression of cancer, and as such have been evaluated as direct and indirect targets for therapeutic intervention, as well as biomarkers for predicting and monitoring treatment responses. (Myatt *et al.*, 2007).

Antibody Production:

Anti-FOXO3a pSer7 (mouse) polyclonal antibody was raised in sheep against FOXO3a pSer7 (residues 3-11 of mouse FOXO3a; Ser7 phosphorylated). The antibodies were purified by the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC-PPU, University of Dundee, Dundee, U.K.) by affinity purification of the anti-FOXO3a pAbs from the sheep serum using a GST-tagged antigen-agarose column. Anti-FOXO3a pSer7 (mouse) pAb was sourced by Ubiquigent directly from the MRC-PPU.

General References:

Anderson M J, Viars CS, Czekay S, Cavenee W K, Arden K C. (1998) Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. *Genomics* **47**, 187-199.

Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME (1999) Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* **96**, 857–68.

Ekoff M, Kaufmann T, Engström M, Motoyama N, Villunger A, Jönsson JI, Strasser A, Nilsson G (2007). The BH3-only protein Puma plays an essential role in cytokine deprivation induced apoptosis of mast cells. *Blood* **110**, 3209–17.

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Skurk C, Maatz H, Kim HS, Yang J, Abid MR, Aird WC, Walsh K (2004). The Akt-regulated forkhead transcription factor FOXO3a controls endothelial cell viability through modulation of the caspase-8 inhibitor FLIP. *J Biol Chem* **279**, 1513–25.

Skurk C, Izumiya Y, Maatz H, Razeghi P, Shiojima I, Sandri M, Sato K, Zeng L, Schiekofer S, Pimentel D, Lecker S, Taegtmeyer H, Goldberg A L, Walsh K (2005) The FOXO3a transcription factor regulates cardiac myocyte size downstream of AKT signaling. *J Biol Chem* **280**, 20814-20823.

Application Reference:

Ho KK, McGuire VA, Koo CY, Muir KW, de Olano N, Maifoshie E, *et al.* (2012) Phosphorylation of FOXO3a on Ser-7 by p38 promotes its nuclear localization in response to doxorubicin. *J Biol Chem* **287**, 1545-1555.

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