Antibody Datasheet

Product description:- Anti-FOXO3a phospho Ser 294

Antigen:- SKWPGS*PTSR

[where * is phospho Serine, residues 289 – 298 of human]

Sheep Number:- S336B

Bleed Number:- Second Bleed

Concentration:- 0.44 mg/ml

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Formulation:- Phosphate Buffered Saline

Storage temperature:- -20 °C

Purification Method:- Affinity purified against phospho peptide

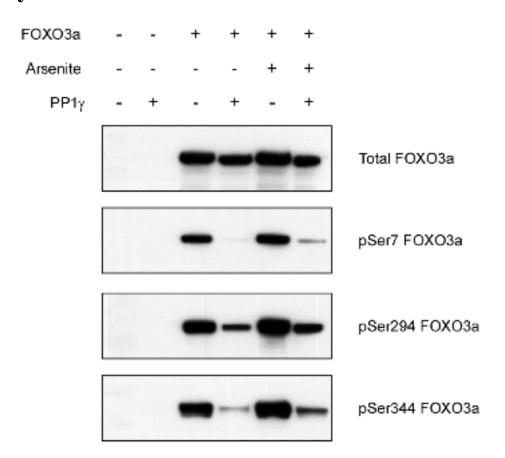
Working Concentration: 1 μ g/ml for immuno blotting after immunoprecipitation

Include the non-phosphorylated form of the peptide immunogen at $10 \mu g/ml$ to the diluted antibody

This antibody only recognises transfected material

Publication Reference:-

Unpublished – Therefore please treat this information as highly confidential



pCMV5-Flag-FOXO3a was transfected into HEK293 cells and cells were serum starved overnight and stimulated with or without arsenite (0.5 M) for 60 mins. Flag-FOXO3a was immunoprecipitated from 100 μ g of cell lysate using an anti-Flag antibody (Sigma). The immunoprecipiate was split into twice, with one half treated with or without 1 mU of PP1 gamma for 30 mins at 30 °C. Samples were subjected to SDS-Page analysis before being transferred to nitrocellulose membrane and immuno blotted with the appropriate phospho specific antibody at 1 μ g/ml or anti-FOXO3a (Upstate).

Binding of the primary antibody was detected using rabbit peroxidase conjugated antisheep IgG antibody (1 in 10, 000 dilution, Pierce) followed by enhanced chemiluminescence (ECL, Amersham).