

AlphaLISA® SureFire® Ultra™

Human and Mouse p-FOXO1 (Ser256) Detection Kit

Product number: ALSU-PFOXO1-A500, ALSU-PFOXO1-A10K,
ALSU-PFOXO1-A50K, ALSU-PFOXO1-A-HV



Kit specificity:

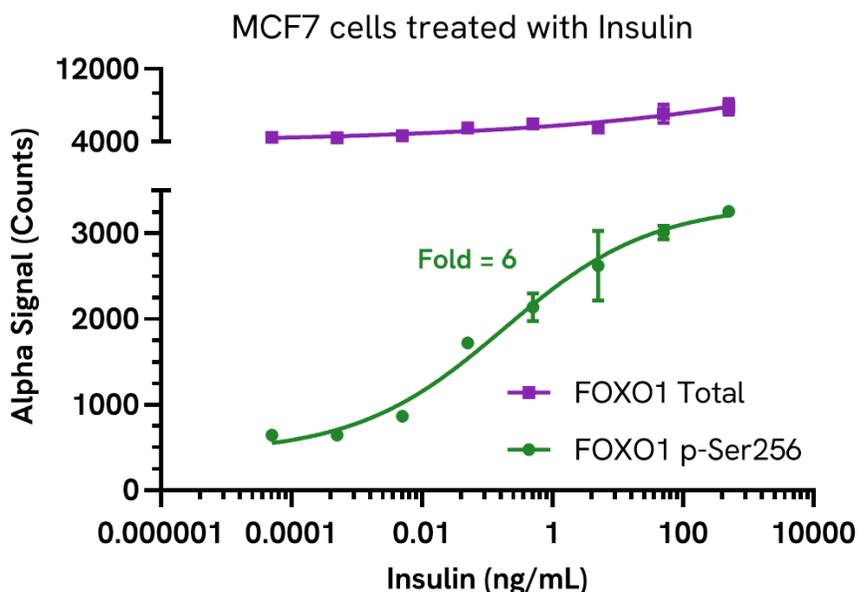
This assay kit contains antibodies which recognize the phospho-Ser256 epitope and a distal epitope on FOXO1. The protein detected by this kit corresponds to UniProt ID Q12778. FOXO1 is also known as Forkhead box protein O1 and Forkhead in rhabdomyosarcoma. These antibodies recognize FOXO1 of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:

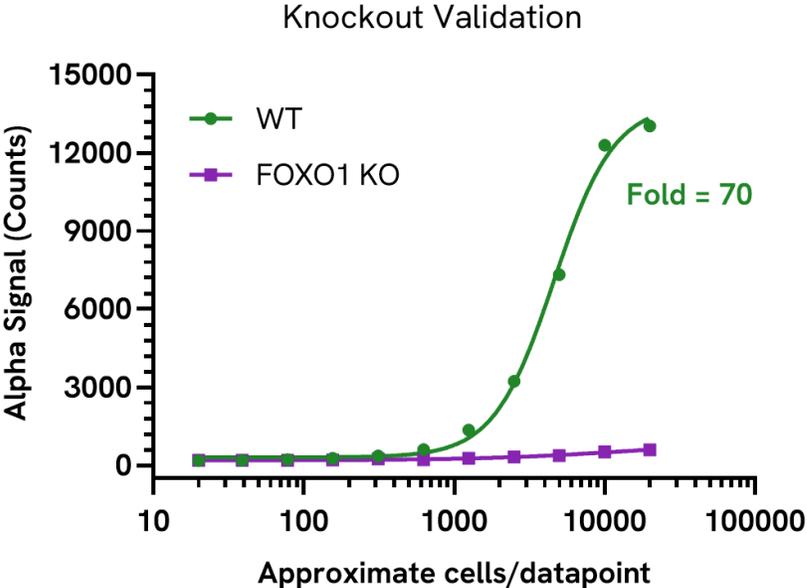
Positive Control Lysate: Prepared from HEK293 cells cultured on confluence in T175 flasks in 10% FBS containing media and lysed in 3 mL of Lysis Buffer.

Representative data:

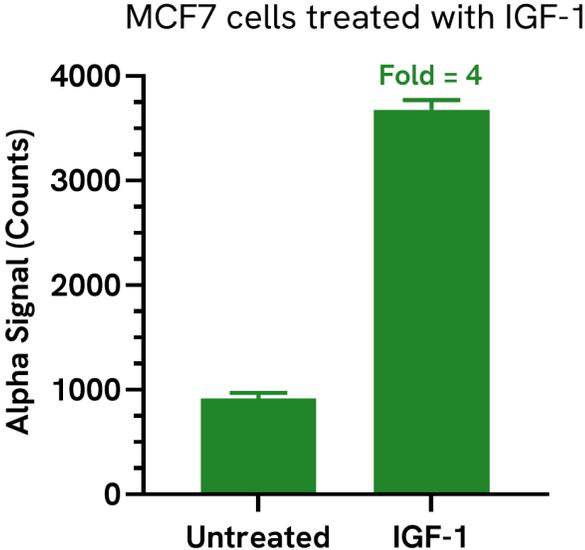
Data obtained with a 2-plate, 2-incubation protocol. MCF7 cells were seeded at 20K cells/well in a 96-well plate and incubated overnight. Cells were starved for 2 hours in 1% FBS containing media, then treated with increasing concentrations of Insulin for 5 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser256) and Total FOXO1 levels using respective *SureFire Ultra* kits. Equivalent to approximately 2,000 cells/datapoint.



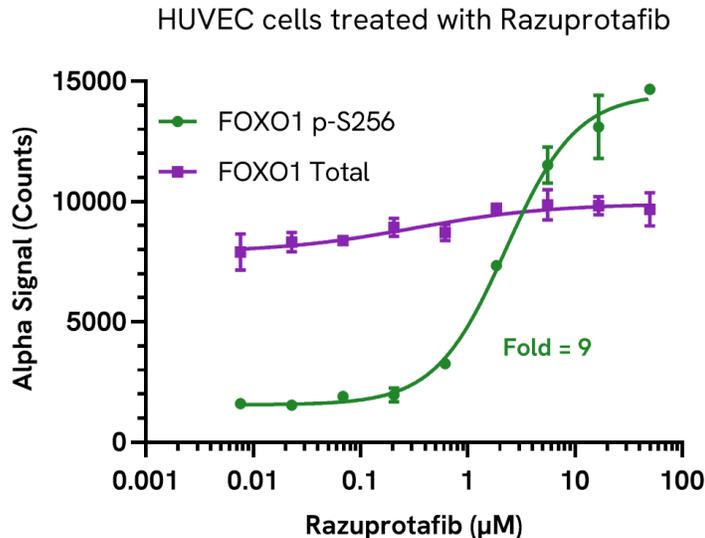
Phospho FOXO1 (Ser256) levels were assessed in HAP-1 wild type (WT) and FOXO1 knockout (KO) cells. Cells were cultured to confluency in T175 flasks and lysed in 4 mL of Lysis Buffer. Lysates were serially diluted in Lysis Buffer and evaluated using the *SureFire Ultra* kit. Phospho FOXO1 (Ser256) was only detected in WT cells, confirming assay specificity.



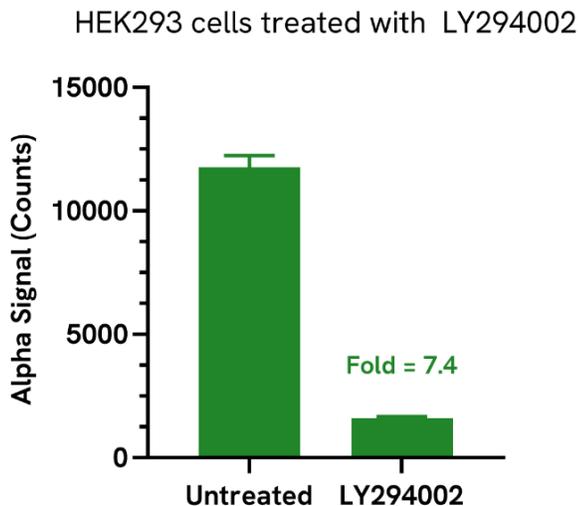
Data obtained with a 2-plate, 2-incubation protocol. MCF7 cells were seeded at 20K cells/well in a 96-well plate and incubated overnight. Cells were starved for 2 hours in 1% FBS containing media then treated with 500 ng/mL insulin growth factor (IGF-1) for 5 minutes. Cells were lysed with Lysis Buffer and assayed for Phospho (Ser256) FOXO1 levels using *SureFire Ultra*. Equivalent to approximately 2,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. HUVEC cells were seeded at 20K cells/well in a 96-well plate and incubated overnight. Cells were starved for 2 hours in 1% FBS containing media, then treated with increasing concentrations of Razuprotafib for 15 minutes. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Ser256) and Total FOXO1 levels using respective *SureFire Ultra* kits. Equivalent to approximately 12,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubated protocol. HEK293 cells were seeded at 20K cells/well in a 96-well plate and incubated overnight. Cells were treated with 50 µM LY294002 for 24 hours. Cells were lysed with Lysis Buffer and assayed for Phospho (Ser256) FOXO1 using *SureFire Ultra*. Equivalent to approximately 2,000 cells/datapoint.



The information provided in this document is for reference purposes only and may not be all-inclusive. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume liability for the accuracy or completeness of the information contained herein. Users should exercise caution when handling materials as they may present unknown hazards. Revvity shall not be liable for any damages or losses resulting from handling or contact with the product, as Revvity cannot control actual methods, volumes, or conditions of use. Users are responsible for ensuring the product's suitability for their specific application. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF"

