AlphaLISA® *SureFire® Ultra™*

Human and Mouse MCL-1 Total Detection Kit

Product number: ALSU-TMCL1-A500, ALSU-TMCL1-A10K, ALSU-TMCL1-A50K, ALSU-TMCL1-A-HV

Kit specificity:

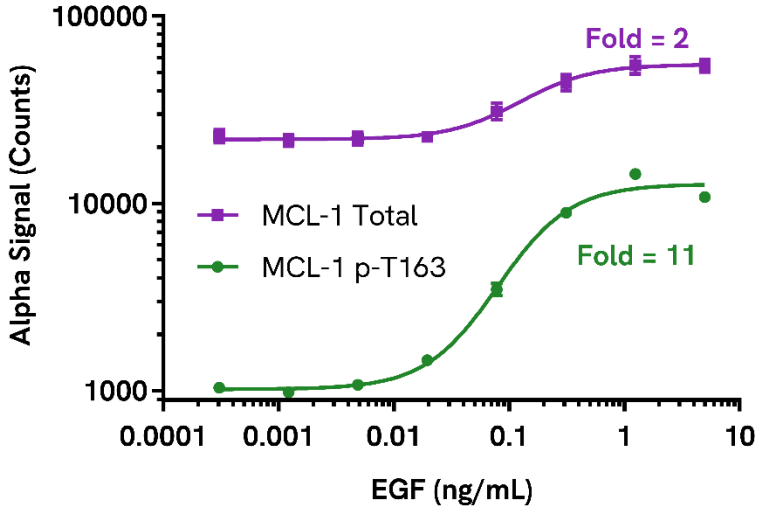
This assay kit contains antibodies which recognize distinct epitopes on MCL-1. The protein detected by this kit corresponds to UniProt ID Q07820. MCL-1 is also known as Bcl-2-like protein 3 (Bcl2-L-3) and Bcl-2-related protein EAT/mcl1. These antibodies recognize MCL-1 of human and mouse origin. Other species should be tested on a case-by-case basis.

Control lysate information:

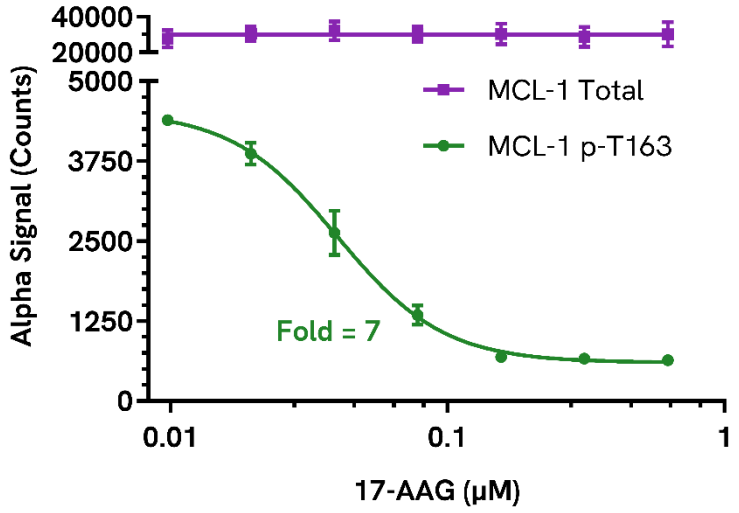
Positive Control Lysate: Prepared from HeLa cells, cultured to confluence in T175 flasks in 10% FBS containing medium. Cells were treated with 50 nM PMA for 30 minutes and lysed with 4 mL of Lysis Buffer.

Representative data:

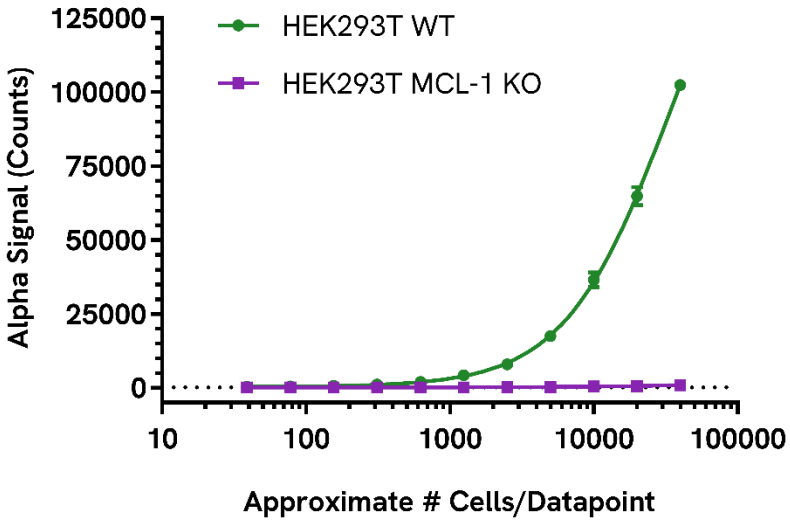
Data obtained with a 2 plate, 2 incubation protocol. HeLa cells were seeded at 20K cells/well in a 96 well plate and incubated overnight. Cells were treated with EGF at the indicated concentrations for 2 hours. Cells were lysed with Lysis Buffer and assayed separately for Phospho (Thr163) and Total MCL-1 using respective *SureFire Ultra* kits. Equivalent to approximately 2,000 cells/datapoint.



Data obtained with a 2 plate, 2 incubation protocol. THP-1 cells were seeded at 150K cells/well in a 96 well plate in complete medium and treated with 17-AAG (HSP90 inhibitor) at the indicated concentrations for 24 hours. Cells were washed in HBSS and lysed with Lysis Buffer and assayed separately for Phospho (Thr163) and Total MCL-1 using respective *SureFire Ultra* kits. Equivalent to approximately 15,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. HEK293T wild type (WT) and HEK293T MCL-1 knockout (KO, Abcam ab266838) cell lines were seeded at increasing cell densities in a 96 well plate and incubated overnight. Cells were lysed with Lysis Buffer and assayed for MCL-1 Total using the *SureFire Ultra* kit.



Data obtained from measurement of MCL-1 Total in various cell types. Adherent cell lines were seeded at 40K cells/well in a 96-well plate and incubated overnight. Cells were lysed with 200 µL of Lysis Buffer. Suspension cell lines were seeded at 400K cells/well in a 96-well plate in HBSS + 0.1% BSA, cells were spun down and lysed with 200 µL of Lysis Buffer. Approximately 2,000 adherent cells or 20,000 suspension cells/datapoint.