

# Technical Data Sheet

## AlphaLISA® SureFire® Ultra™ p-MLKL (Ser358) Assay Kit

ALSU-PMLKL-B500, ALSU-PMLKL-B10K, ALSU-PMLKL-B50K, ALSU-PMLKL-B-HV

### Kit Specificity

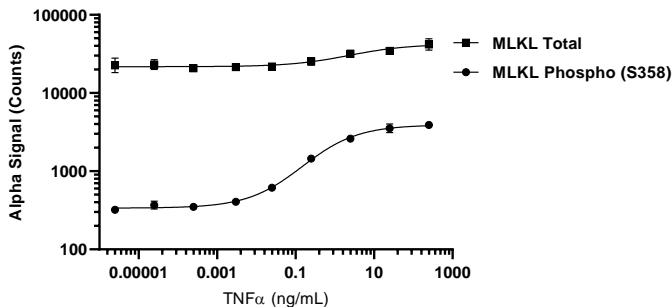
This assay kit contains antibodies which recognize the phospho-Ser358 epitope and a distal epitope on MLKL. The protein detected by this kit corresponds to UniProt ID Q8NB16. MLKL is also known as Mixed lineage kinase domain-like protein. These antibodies recognize MLKL of human origin. Other species should be tested on a case-by-case basis.

### Control Lysate Information

Positive control lysate: Prepared from U937 cells cultured in T175 flasks to  $10^6$  cells/mL in RPMI containing 10% FBS. Cells were treated with 20  $\mu$ M Z-VAD for 30 minutes at 37°C, and then treated with 100 nM SM-164 and 20 ng/mL TNF $\alpha$  for 4 hours at 37°C. Total stimulus time 4.5 hours. Cells were then washed with HBSS and lysed in 5 mL 1X *SureFire Ultra* Lysis buffer.

### Representative Data

Data obtained with a 2-plate, 2-incubation protocol. U937 cells were plated at 300K cells per well in a 96 well plate in RPMI + 10% FBS, then treated with 20  $\mu$ M Z-VAD for 30 minutes at 37°C. Then cells were treated with 100 nM SM-164 and TNF $\alpha$  at the indicated concentrations for 4 hours at 37°C. Cells were then washed with HBSS, lysed with 1X Lysis buffer, and assayed for phospho (Ser358) or Total MLKL in separate wells of an assay plate with respective *SureFire Ultra* kits. Equivalent to approximately 15,000 cells/datapoint.



For more information and detailed protocols, please refer to the generic AlphaLISA *SureFire Ultra* Assay Kits Manual, available from <http://www.perkinelmer.com/category/alpha-surefire-kits>