AlphaLISA® *SureFire® Ultra™*

Human p-PKR (Thr446) Detection Kit

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

Kit specificity:

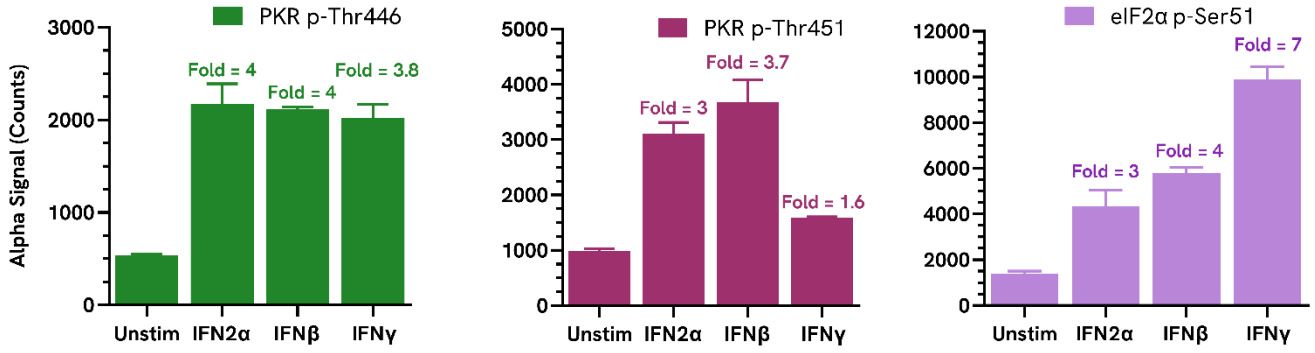
This assay kit contains antibodies which recognize the phospho-Thr446 epitope and a distal epitope on PKR. The protein detected by this kit corresponds to UniProt ID P19525. PKR is also known as Interferon-induced, double-stranded RNA-activated protein kinase and eIF-2A protein kinase 2. These antibodies recognize PKR of human origin. Other species should be tested on a case-by-case basis.

Control lysate information:

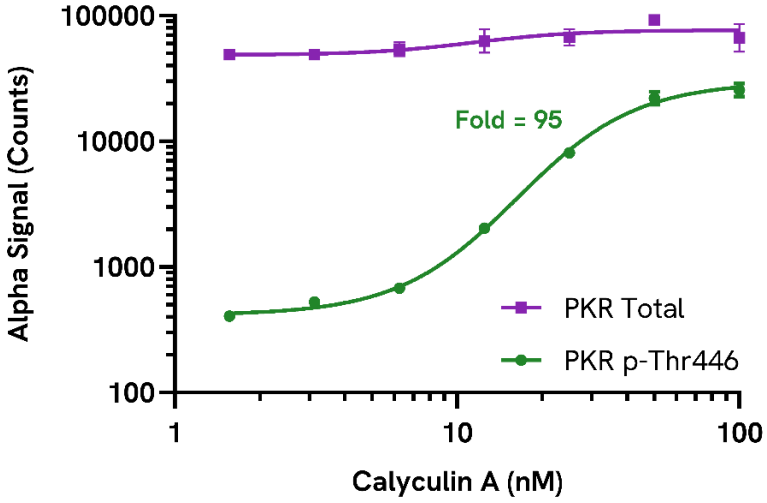
Positive Control Lysate: Prepared from HeLa cells, cultured to confluence in T175 flasks in 10% FBS containing media, then treated with 50 ng/mL Calyculin A for 45 minutes and lysed with 4 mL of Lysis Buffer.

Representative data:

Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded in a 12-well plate at 250,000 cells/well in medium containing 100 nM PMA and incubated for 24 hours. After 24 hours of pretreatment, the THP-1 differentiated macrophages were treated with 250 ng/mL of IFNa, IFNB or IFNy for a further 24 hours. After treatment, cells were washed with HBSS and lysed with Lysis Buffer. PKR Phospho (Thr446 and Thr451) and eIF2a Phospho (Ser51) levels were evaluated using respective *SureFire Ultra* kits. Equivalent to approximately 25,000 cells/datapoint.



Data obtained with a 2-plate, 2-incubation protocol. HeLa cells were seeded in a 96-well plate at 20,000 cells/well in complete medium and incubated overnight. The cells were pretreated with 10 ng/mL IFNγ for 24 hours, then treated with Calyculin A at the indicated concentrations for 30 minutes. After treatment, the cells were lysed with 100 µL of Lysis Buffer and assayed for PKR Phospho (Thr446) using the *SureFire Ultra* kit. Equivalent to approximately 2,000 cells/datapoint.



Specificity of the Phospho (Thr446) PKR assay was assessed by a peptide competition assay. Phospho (Thr446 and Thr451) and Non-Phospho PKR peptides were serially diluted into a fixed concentration of THP1 positive control lysate. Lysates were then assayed using the *SureFire Ultra* PKR Phospho (Thr446) kit.