

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:

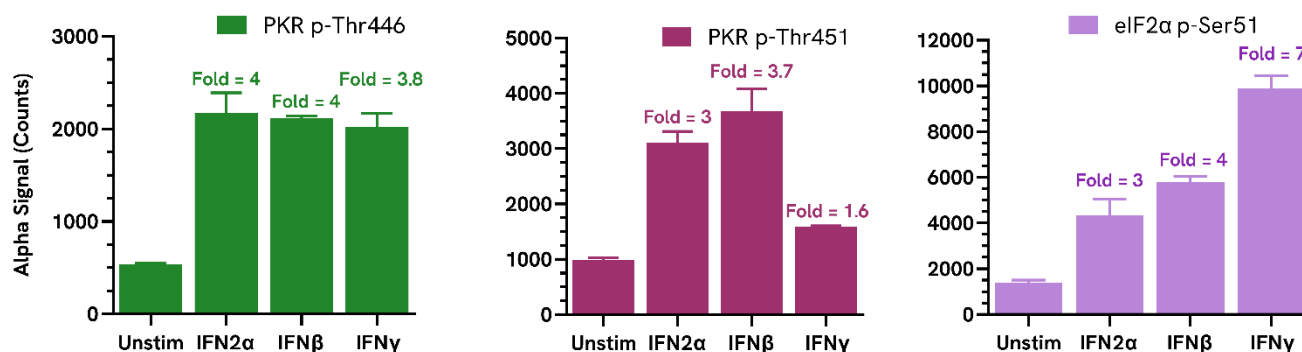
This assay kit contains antibodies which recognize the phospho-Thr446 epitope and a distal epitope on PKR. The protein detected by this kit corresponds to UniProt ID P19525. PKR is also known as Interferon-induced, double-stranded RNA-activated protein kinase and eIF-2A protein kinase 2. These antibodies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

Control lysate information:

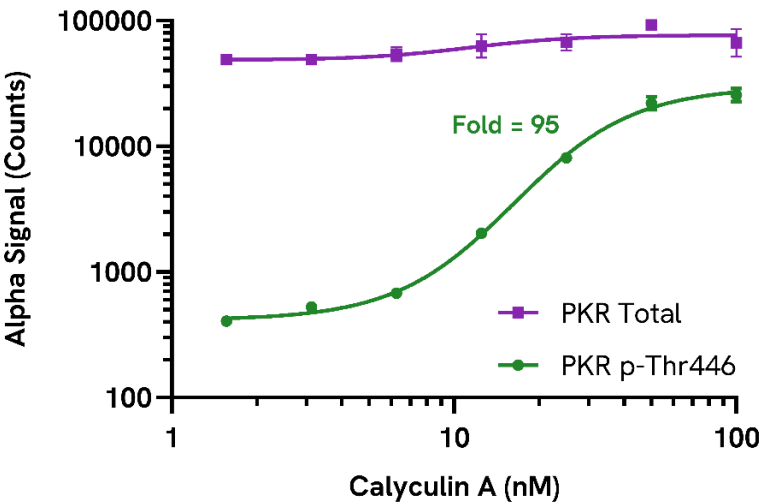
Positive Control Lysate: Prepared from HeLa cells, cultured to confluence in T175 flasks in 10% FBS containing media, then treated with 50 ng/mL Calyculin A for 45 minutes and lysed with 4 mL of Lysis Buffer.

Representative data:

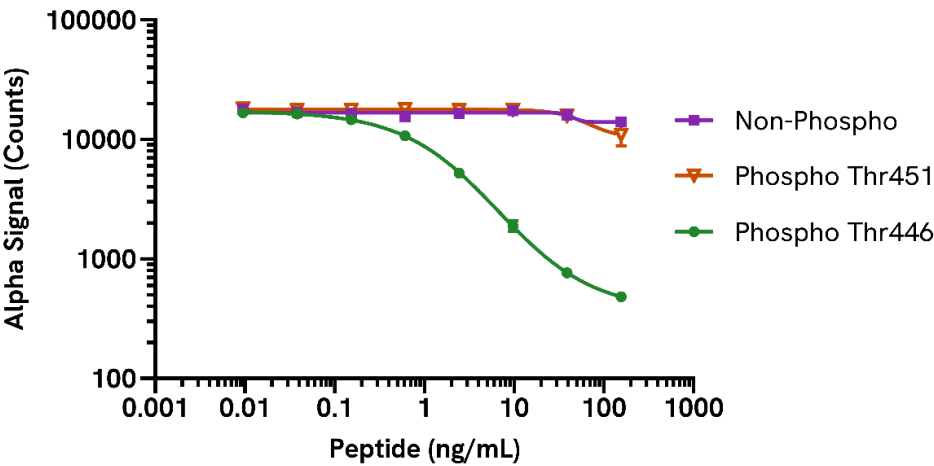
Data obtained with a 2-plate, 2-incubation protocol. THP-1 cells were seeded in a 12-well plate at 250,000 cells/well in medium containing 100 nM PMA and incubated for 24 hours. After 24 hours of pretreatment, the THP-1 differentiated macrophages were treated with 250 ng/mL of IFN α , IFN β or IFN γ for a further 24 hours. After treatment, cells were washed with HBSS and lysed with Lysis Buffer. PKR Phospho (Thr446 and Thr451) and eIF2 α 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 IFN γ for 24 hours, then treated with Calyculin A at the indicated concentrations for 30 minutes. After treatment, the cells were lysed with 100 μ L 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.



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"

