Human PKR/EIF2AK2 Rabbit Polyclonal Antibody

Cat# CY-P1043

100 µg (1.0 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
-	IP, E	H	66 kDa	Rabbit IgG

Background:

The double-stranded RNA-activated protein kinase (PKR), also known as eukaryotic translation initiation factor 2-alpha kinase 2 (EIF2AK2), is a ubiquitously expressed serine/threonine protein kinase that plays a key role in the innate immunity response to viral infection in higher eukaryotes and has also been implicated in several cellular signal transduction pathways (1-4).

The dsRNAs-mediated activation leads to autophosphorylation of PKR and allows the kinase to phosphorylate its natural substrate, the α-subunit of initiation factor eIF2, resulting in rapid inhibition of translation and suppression of virus spread (5, 6). PKR also has been implicated in regulating other cellular functions such as differentiation (7), transcription (8, 9), signal transduction (10), cell growth (11, 12) and apoptosis in the event of virus infection and other forms of cellular stress. (13).

It was reported that the activation of PKR in adipose and liver tissue is caused by obesity (14). In the absence of PKR, metabolic deterioration due to excess energy or nutrition is alleviated. These findings demonstrate that PKR is an important component of inflammation complex that responds to nutrients and organelle dysfunction.

Specificity/Sensitivity: The antibody detects endogenous levels of PKR/EIF2AK2 protein.

Source/Purification: The antibody is produced by immunizing rabbit with a synthetic peptides corresponding to middle region of human PKR. IgG is purified by immunoaffinity chromatography.

Recommended Antibody Dilutions: IP-kinase assay: 1-2 µg/sample.

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50% glycerol. Store at -20°C.

Applications Key: WB: Western **IP:** Immunoprecipitation **IHC:** Immunohistochemistry **IC:** Immunocytochemistry **F:** Flow cytometry **E:** ELISA **FP:** Fluorescence Polarization assay

Species Cross-Reactivity Key: H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Mi:** mink **C:** chicken **X:** Xenopus **Z:** zebra fish **All:** all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

General References:

1. Clemens MJ, Elia A. The double-stranded RNA-dependent protein kinase PKR: structure and function. J Interferon Cytokine Res 17, 503–524, 1997

2. Kaufman, RJ. The double stranded RNA-activated protein kinase PKR. In: Sonenberg, N. et al., editors. Translational Control of Gene Expression. Cold Spring Harbor Laboratory Press; 2000. p503-528, 2000.

3. Robertson HD, Mathews MB. The regulation of the protein kinase PKR by RNA. Biochimie 78, 909–914, 1996

4. Williams BR. Signal integration via PKR. Sci STKE RE2, 2001

5. Stark GR, Kerr IM, Williams BRG, Silverman RH and Schreiber RD. How cells respond to interferons. Annu Rev Biochem, 67, 227–264, 1998

6. Williams BRG. PKR; a sentinel kinase for cellular stress. Oncogene, 18, 6112–6120, 1999

7. Judware, R., and Petryshyn, R. Mechanism of action of a cellular inhibitor of the dsRNA-dependent protein kinase from 3T3-F442A cells. J. Biol. Chem. 267, 21685–21690, 2992

8. Kumar, A., Yang, Y. L., Flati, V., Der, S., Kadereit, S., Deb, A., Haque, J., Reis, L., Weissmann, C., and Williams, B. R. Deficient cytokine signaling in mouse embryo fibroblasts with a targeted deletion in the PKR gene: role of IRF-1 and NF-kappaB, EMBO J. 16, 406–416, 1997

9. Kumar, A., Haque, J., Lacoste, J., Hiscott, J., and Williams, B. R. Double-stranded RNA-dependent protein kinase activates transcription factor NF-kB by phosphorylating IkB. Proc.Natl. Acad. Sci. U. S. A. 91, 6288–6292, 1994

10. Yang, Y., Reis, L. F. L., Pavlovic, J., Aguzzi, A., Schafer, R., Kumar, A., Williams, B. R. G., Aguet, M., and Weissmann, C. Deficient signaling in mice devoid of double-stranded RNA-dependent protein kinase. EMBO J. 14, 6095–6106, 1995

11. Meurs, E. F., Galabru, J., Barber, G. N., Katze, M. G., and Hovanessian, A. G. Tumor suppressor function of the interferon-induced double-stranded RNA-activated protein kinase. Proc. Natl. Acad. Sci. U. S. A. 90, 232–236, 1993

12. Barber, G. N., Wambach, M., Thompson, S., Jagus, R., and Katze, M. G. Mutants of the RNA-dependent protein kinase (PKR) lacking double-stranded RNA binding domain I can act as transdominant inhibitors and induce malignant transformation. Mol. Cell. Biol. 15, 3138–3146, 1995

13 Takizawa, T., Ohashi, K., and Nakanishi, Y. Possible involvement of double-stranded RNA-activated protein kinase in cell death by influenza virus infection. J. Virol. 70, 8128–8132, 1996

14. Nakamura T, Furuhashi M, Li P, Cao H, Tuncman G, Sonenberg N, Gorgun CZ, Hotamisligil GS.; Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. Cell. 140, 338-48, 2010

Immunoprecipitation Protocol Followed by Measuring PKR Activity:

Preparation of Solution and Reagent

1. Cell Lysis Buffer
   20 mM Tris HCl, pH 7.5, 250 mM NaCl, 10% glycerol, 0.5% Nonidet® P-40, 1 mM EDTA, 1 mM EGTA, 0.2 mM PMSF, 1 µg/mL pepstatin, 0.5 µg/mL leupeptin, 5 mM NaF, 2 mM Na_3VO_4, 2 mM β-glycerophosphate, 1mM DTT, 1x Phosphatase Inhibitor Cocktail
2. Protein A Agarose Beads (50% bead slurry)
   Add 5 mL of 1X PBS to 1.5 g of protein A agarose beads. Shake 2 hours at 4°C; spin down. Wash beads twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C)
3. Poly-L-lysine-coated Plate
   Coat the plate with 25 µg/mL poly-L-lysine (PLL) in PBS for 4-12 h at 37°C. Subsequent to a washing step with PBS.

Treatment of Cells

1. Plate adherent cells in PLL-coated 6 well-plate dish at ~1 x 10^6 cells/plate.
2. Incubate the culture dish at 37°C for 12-16 hours in CO_2 incubator.
3. Change the medium to fresh media containing 10 µg/mL poly I:C.
4. Incubate the culture dish at 37°C for appropriate time.

Preparing Cell Lysates

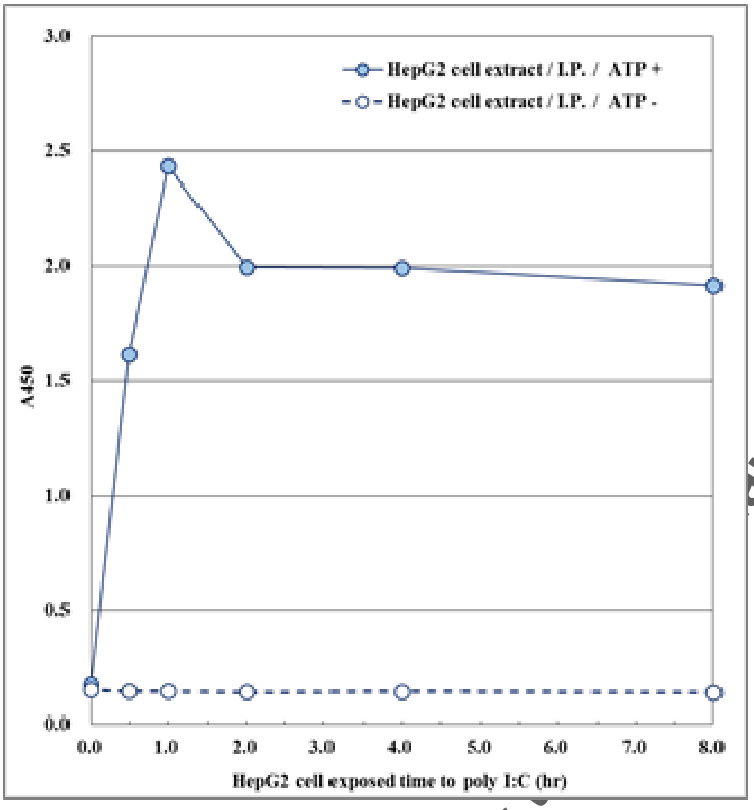
1. Remove media, and wash cells with ice-cold PBS and aspirate.
2. Add 1 mL of ice-cold Cell Lysis Buffer to each plate (6-well plate) and shake at ca. 300 rpm on an orbital microplate shaker for 120 minutes each at 4°C.
3. Scrape off and transfer the lysate to microcentrifuge tubes and microcentrifuge at 15, 000 rpm for 10 minutes at 4°C,
4. Transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, the lysate can be stored at -70°C.

Immunoprecipitation and assay kinase activity

1. Take 100 µL of the cell lysate and add anti-human PKR polyclonal antibody (Cat#CY-P1043; 1-2 µg) and incubate with gentle rocking for 1–3 hours at 4°C.
2. Add 20 µL of protein A agarose beads (50% bead slurry) and incubate with gentle rocking for 1-3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash the protein A agarose beads 3 times with 500 µL of Cell Lysis Buffer and once with Kinase Buffer. Keep on ice during the washes.
4. Re-suspend the protein A agarose beads with 20-40 µL of Kinase Buffer and use 10 µL as an enzyme sample* to measure PKR activity using CycLex PKR/EIF2AK2 Kinase Assay Kit (Cat# CY-1184)

* Please take care to transfer the protein A agarose beads to the well of the Antibody-coated Microplate as little as possible.

Fig.1 Time course of poly I:C effect on the kinase activity of PKR in HepG2 cell, measured by IP-kinase aaasay using CycLex PKR/EIF2AK2 Kinase Assay Kit (Cat#CY-1184) and the human PKR/EIF2AK2 rabbit polyclonal antibody (Cat#CY-P1043)



Related Products:

*** CycLex PKR/EIF2AK2 Kinase Assay Kit: Cat#CY-1184**
*** PKR/EIF2AK2 (Human), Active: Cat#CY-SPP80**
*** PKR/EIF2AK2 Positive Control (Full length): Cat#CY-E1184-1**
*** Anti-Human PKR/EIF2AK2 Polyclonal Antibody: Cat#CY-P1043**

PRODUCED BY

CycLex Co., Ltd.
1063-103 Terasawaoka
Ina, Nagano 396-0002
Japan
Fax: +81-265-76-7618
E-mail: info@cyclex.co.jp
URL: http://www.cyclex.co.jp