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ProductInformation

Anti-phospho-eIF-2α [$pSer^{52}$]
produced in rabbit, affinity isolated antibody

Catalog Number **E 2152**

Product Description

Anti-phospho-eIF-2α [$pSer^{52}$] is developed in rabbit using a synthetic phosphopeptide derived from a region of human eIF-2α containing serine 52 as the immunogen. This region is identical among many species, including human, rat, pig and yeast. The serum is affinity purified using epitope-specific affinity chromatography, and is preadsorbed to remove any reactivity towards the non-phosphorylated eIF-2α protein.

Anti-phospho-eIF-2α [$pSer^{52}$] is specific for the phosphorylated form of the α subunit of eukaryotic translation initiation factor 2 (eIF-2α) containing a phosphate on serine 52. It recognizes human, mouse and yeast eIF-2α. It does not cross-react with non-phosphorylated eIF-2α or eIF-2α phosphorylated at other sites. The antibody has been used in Western blotting and immunostaining.

The eIF-2 protein is expressed in many cell types. It is composed of α, β and γ subunits and plays a key role in the initiation of translation.[1, 2] eIF-2 forms a complex with GTP and initiator tRNA that binds to a 40S ribosomal subunit. A 43S preinitiation complex is formed upon the binding of mRNA. The 80S initiation complex is formed when the 60S ribosomal subunit joins the complex following the hydrolysis of the GTP bound to eIF-2 and release of the eIF-2 -GDP. The recycling of eIF-2 requires the exchange of the bound GDP for GTP by a reaction catalyzed by eIF-2B.

Both rat and human eIF-2α contain 315 amino acids (36.1 kDa).[1] In mammalian cells, it is phosphorylated at serine 52 by two kinases: repressor HCR and the interferon inducible double-stranded RNA-dependent protein kinase (PKR).[3] Phosphorylation of eIF-2α blocks the GDP-GTP exchange activity of eIF-2B resulting in the suppression of protein synthesis.[4, 5]

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

Storage/Stability

Store at –20 °C. Due to the presence of 50% glycerol the antibody will remain in solution. For extended storage, centrifuge the vial briefly before opening and prepare working aliquots. To ensure accurate dilutions mix gently, remove excess solution from pipette tip with clean absorbent paper, pipette slowly. The antibody is stable for at least six months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Product Profile

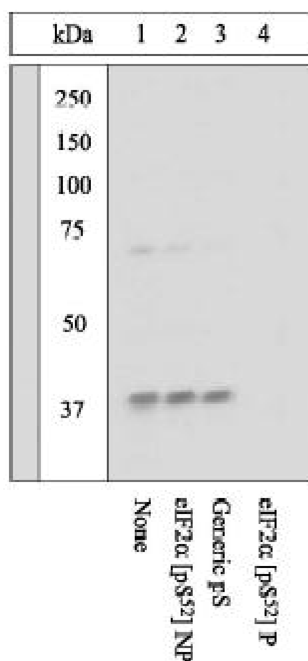
The recommended working dilution of 1: 1000 is determined by immunoblotting using 3T3-L1 adipocytes stimulated with Leukemia Inhibitory Factor (LIF).

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Peptide Competition

1. Extracts prepared from 3T3-L1 adipocytes treated with LIF were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% non-fat dried milk-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:
   Lane 1 no peptide
   Lane 2 non phosphorylated peptide corresponding to the immunogen
   Lane 3 a generic phosphoserine containing peptide
   Lane 4 immunogen

4. After preincubation membranes were incubated with anti-phospho-eIF-2α (pSer[52]) antibody for two hours at room temperature in a 3% non-fat dried milk-TBST buffer.
5. After washing, membranes were incubated with goat $F(ab')_2$ anti-rabbit IgG-HRP and signals were detected using Pierce SuperSignal® method.



The data show that only the peptide corresponding to eIF-2α (pSer[52]) blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

1. Ernst, H. et al., Cloning and sequencing of complementary DNAs encoding the α-subunit of translational initiation factor eIF-2. Characterization of the protein and its messenger RNA. *J. Biol. Chem.*, **262,** 1206-1212 (1987).
2. Tan, S., et al., Regulation of antioxidant metabolism by translation initiation factor 2α. *J. Cell Biol.*, **152,** 997-1006 (2001).
3. Lu, J., et al., The interferon-induced double-stranded RNA-activated protein kinase PKR will phosphorylate serine, threonine, or tyrosine at residue 51 in eukaryotic initiation factor 2α., J*. Biol. Chem.*, **274,** 32198-32203 (1999).
4. Datta, B., et al., Increased phosphorylation of eukaryotic initiation factor 2α at the G2/M boundary in human osteosarcoma cells correlates with deglycosylation of p67 and a decreased rate of protein synthesis. *Exp. Cell Res.*, **250,** 223-230 (1999).
5. Pavitt, G.D., et al., eIF2 independently binds two distinct eIF2B subcomplexes that catalyze and regulate guanine-nucleotide exchange. *Genes Dev.*, **12,** 514-526 (1998).

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