3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-phospho-eIF2Bε [pSer^{539}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **E7655**

Product Description

Anti-phospho-eIF2Bε (Eukaryotic Initiation Factor 2B) [pSer^{539}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of eIF2Bε that contains serine 539 as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated peptide. Anti-phospho-eIF2Bε [pSer^{539}] specifically recognizes eIF2Bε phosphorylated at serine 539.The antibody detects human and rat eIF2Bε. Other species have not been tested. It has been used in immunoblotting applications.[1]

Eukaryotic Initiation Factor 2B (eIF2B) plays a key role by acting as a guanine nucleotide exchange factor in the initiation of mRNA translation in eukaryotic cells. The activity of eIF2B is regulated by a wide array of conditions. Stress inhibits its action, while stimulation of protein synthesis results in activation.

eIF2Bε, an 82 kDa subunit of eIF2B, is phosphorylated at serine 539 by glycogen synthase kinase-3 (GSK-3), which leads to inhibition of its guanine nucleotide exchange activity.

eIF2Bε, an 82 kDa subunit of eIF2B, is phosphorylated at serine 539 by glycogen synthase kinase-3 (GSK-3), which leads to inhibition of its guanine nucleotide exchange activity.

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.

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.

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. The antibody is stable for at least six months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

A recommended working concentration of 0.1 to 1.0 µg/mL is determined by immunoblotting using recombinant eIF2Bε treated with GSK-3β and lambda (λ) phosphatase, CHO-T cells transfected with human insulin receptor (IR) +/- insulin.

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

Results

Peptide Competition

1. Recombinant eIF-2Bε treated with lambda phosphatase λ, (lane 1) or treated with GSK-3β (lanes 2-5) and added to background extracts were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:

Lanes 1 and 2	no peptide
Lane 3	non-phosphorylated peptide corresponding to the immunogen
Lane 4	a generic phosphoserine containing peptide
Lane 5	immunogen

4. All lanes were incubated with 0.50 µg/mL eIF-2Bε [pSer^{539}] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

The data in Figure 1 shows that only the peptide corresponding to eIF-2Bε [pSer^{539}] blocks the antibody signal, demonstrating the specificity of the antibody

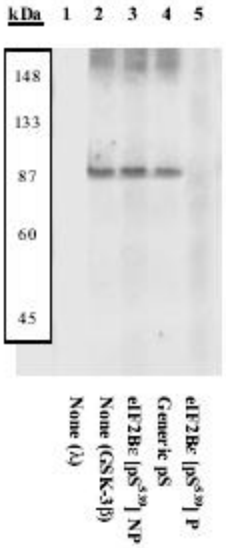

Figure 1 Peptide Competition

.

References

1. Vary, T.C., et al. Phosphorylation of eukaryotic initiation factor eIF2B epsilon in skeletal muscle during sepsis. Am. J. Physiol.- Endocrinology and Metabolism, **283**, E1032-E1039 (2002).
2. Wang, X., et al. Eukaryotic initiation factor 2B: identification of multiple phosphorylation sites in the epsilon-subunit and their functions in vivo. EMBO J. 20(16):4349-4359 (2001).
3. Woods, Y.L., et al. The kinase DYRK phosphorylates protein-synthesis initiation factor eIF2Bepsilon at Ser539 and the microtubule-associated protein tau at Thr212: potential role for DYRK as a glycogen synthase kinase 3-priming kinase. Biochem. J., **355**, 609-615 (2001).
4. Kleijn, M., and C.G. Proud The activation of eukaryotic initiation factor (eIF)2B by growth factors in PC12 cells requires MEK/ERK signalling. FEBS Lett., **476**, 262-265 (2000).
5. Jefferson, L.S., et al. Glycogen synthase kinase-3 is the predominant insulin-regulated eukaryotic initiation factor 2B kinase in skeletal muscle. Int. J. Biochem., Cell. Biol. **31**, 191-200 (1999).

AH/JK 5/23/2004