



## Product Information

### Anti-phospho-eIF4G [pSer<sup>1108</sup>]

Developed in Rabbit, Affinity Isolated Antibody

Product Number **E 7530**

#### Product Description

Anti-phospho-eIF4G (Eukaryotic Initiation Factor 4G) [pSer<sup>1108</sup>] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human eIF4G that contains serine 1108 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.

The antibody detects human eIF4G. Rabbit (92% homologous) has not been tested but is expected to cross react. It has been used in immunoblotting applications.

Eukaryotic initiation factor 4G (eIF4G) is a member of the eIF4 class of translational initiation factors involved in recognition of the mRNA cap, ATP-dependent unwinding of 5'-terminal secondary structure, and recruitment of mRNA to the ribosome. eIF4G is a 200 kDa scaffolding protein that binds eIF4E, eIF4A, and the eIF4E-kinase Mnk1 simultaneously.

The C-terminus of eIF4G is highly phosphorylated and contains three serine phosphorylation sites (1108, 1148 and 1192). eIF4G serine 1108 is phosphorylated in response to various extracellular stimuli including serum, and is sensitive to specific inhibitors of the PI3K and FRAP/mTOR signaling pathways.

#### Reagent

Anti-phospho-eIF4G [pSer<sup>1108</sup>] is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg<sup>2+</sup> and Ca<sup>2+</sup>), pH 7.3, with 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 -70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples 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 0.5 to 1.5 µg/mL is determined by immunoblotting using Hek293 cells.

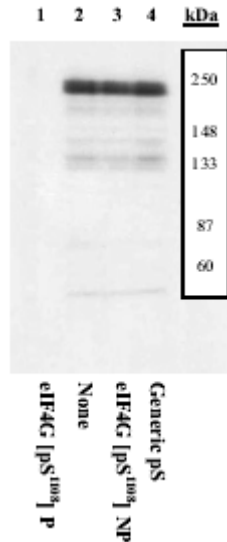
**Note:** In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

#### Results

##### Peptide Competition

1. Extracts prepared from Hek cells were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 3% non-fat milk-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:  
Lane 1 immunogen  
Lane 2 no peptide  
Lane 3 non-phosphorylated peptide corresponding to the immunogen  
Lane 4 a peptide containing generic phosphoserine.
4. All lanes were incubated with 1.0 µg/mL eIF4G [pSer<sup>1108</sup>] antibody for two hours at room temperature in a 3% non-fat milk-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')<sub>2</sub> anti-rabbit IgG alkaline phosphatase and signals were detected

The data in Figure 1 show that only the peptide corresponding to eIF4G [pSer<sup>1108</sup>] blocks the antibody signal, thereby demonstrating the specificity of the antibody.



**Figure 1 Peptide Competition**

## References

1. DeGracia, D.J., et al., Molecular pathways of protein synthesis during brain reperfusion: implications for neuronal survival or death. *J. Cereb. Blood Flow Metab.*, **22**, 127-141 (2002).
2. Bolster, D.R., et al. AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J. Biol. Chem.*, **277**, 23977-23980 (2002).
3. Raught, B., et al. Serum-stimulated, rapamycin-sensitive phosphorylation sites in the eukaryotic translation initiation factor 4G1. *EMBO J.*, **19**, 434-444 (2000).
4. Pyronnet, S., et al. Human eukaryotic translation initiation factor 4G (eIF4G) recruits mnk1 to phosphorylate eIF4E. *EMBO J.*, **18**, 270-279 (1999).

AH/JK 4/16/2004

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