

Gcn4 (FL-281): sc-50443

BACKGROUND

During amino acid starvation, *Saccharomyces cerevisiae* utilizes a network of proteins to activate the transcription of amino acid biosynthetic genes. Phosphorylation of the eukaryotic initiation factor 2 (eIF2 α) by Gcn2 downregulates total protein synthesis as well as decreases the amount of uncharged tRNA. Uncharged tRNA recognizes the initiation codon of Gcn4 mRNA to increase levels of the Gcn4 protein, a transcriptional activator of amino acid biosynthetic precursors. Gcn4 recognizes a specific DNA-binding motif sequence designated the Gcn4-protein responsive element (GCRE), which is present in the promoter regions of its target genes. Gcn4 (31 kDa) targets include genes in every amino acid biosynthetic pathway except cysteine as well as genes encoding vitamin biosynthetic enzymes, peroxisomal components, mitochondrial carrier proteins and autophagy proteins. UV radiation and glucose stimulation also induce Gcn4 activity through the Ras/cAMP pathway.

REFERENCES

- Hinnebusch, A.G. 1993. Gene-specific translational control of the yeast Gcn4 gene by phosphorylation of eukaryotic initiation factor 2. *Mol. Microbiol.* 10: 215-223.
- Natarajan, K., et al. 2001. Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during amino acid starvation in yeast. *Mol. Cell. Biol.* 21: 4347-4368.
- Marbach, I., et al. 2001. Gcn2 mediates Gcn4 activation in response to glucose stimulation or UV radiation not via Gcn4 translation. *J. Biol. Chem.* 276: 16944-16951.
- Grundmann, O., et al. 2001. Repression of Gcn4 mRNA translation by nitrogen starvation in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 276: 25661-25671.
- Lu, S.M. and Hodges, R.S. 2002. A *de novo* designed template for generating conformation-specific antibodies that recognize α -helices in proteins. *J. Biol. Chem.* 277: 23515-23524.
- Kubota, H., et al. 2003. Rapamycin-induced translational derepression of Gcn4 mRNA involves a novel mechanism for activation of the eIF2 α kinase Gcn2. *J. Biol. Chem.* 278: 20457-20460.

SOURCE

Gcn4 (FL-281) is a rabbit polyclonal antibody raised against amino acids 1-281 representing full length Gcn4 of *Saccharomyces cerevisiae* origin.

PRODUCT

Each vial contains 200 μ g IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

STORAGE

Store at 4 $^{\circ}$ C, ****DO NOT FREEZE****. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

Gcn4 (FL-281) is recommended for detection of Gcn4 of *Saccharomyces cerevisiae* origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 μ g per 100-500 μ g of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Molecular Weight of Gcn4: 31 kDa.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz MarkerTM compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz MarkerTM Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruzTM Mounting Medium: sc-24941.

SELECT PRODUCT CITATIONS

- Smets, B., et al. 2008. Genome-wide expression analysis reveals TORC1-dependent and -independent functions of Sch9. *FEMS Yeast Res.* 8: 1276-1288.
- Kimura, A., et al. 2012. N-myristoylation of the Rpt2 subunit regulates intracellular localization of the yeast 26S proteasome. *Biochemistry* 51: 8856-8866.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.