

Product Information

Monoclonal Anti-PSF antibody produced in mouse clone B92, purified from hybridoma cell culture

Catalog Number **P2860**

Product Description

Monoclonal Anti-PSF (mouse IgG1 isotype) is derived from the B92 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a C57BL/6J mouse immunized with a lysate of mouse bone marrow-derived stromal cell line.¹ The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-PSF reacts specifically with PSF (polypyrimidine tract-binding protein-associated splicing factor). The antibody is useful in immunoblotting,¹⁻³ immunoprecipitation,^{1,2} RIA (of cells),¹ affinity chromatography,¹⁻³ immunocytochemistry (2 min in 3% paraformaldehyde with 0.5% Triton X-100, and then 20 min in 3% paraformaldehyde)¹ and immunohistochemistry (frozen sections, 4% paraformaldehyde-0.5% Triton X-100-treated). By immunoblotting of mouse cells of immature myeloid origin (bone marrow and acute myeloid leukemia), the antibody identifies a 47 kDa protein, with an apparent M.W. of 49 kDa.³ In lymphoid cells, peripheral blood cells and other tissues, it recognizes a major 100 kDa antigen and a cleavage product of 68 kDa.³ In HeLa cells, bands of 120, 100³, 80 and 68³ kDa are stained by the antibody. It stains immature granulocyte nuclei in a speckled pattern characteristic of pre-mRNA splicing factors.^{1,3} Reactivity has been observed with human³ and mouse¹⁻³ PSF.

The RNAs that direct protein synthesis in animals and plant cells are synthesized in the nucleus as large precursors (pre-mRNAs). The protein coding sequences in pre-mRNA molecules are arranged in discontinuous segments - exons - interspersed with noncoding sequences - introns. In a process termed splicing, these introns are efficiently removed before the pre-mRNA is transported from the nucleus to the cytoplasm, where it is translated into protein.⁴ Studies have shown that nuclear pre-mRNA splicing takes place in a multi-component structure termed a

spliceosome. The polypyrimidine tract-binding (PTB) protein-associated splicing factor (PSF), which plays an essential role in mammalian spliceosomes, is a ubiquitous nuclear matrix protein.⁵ A complex between PTB and PSF is necessary for pre-mRNA splicing. PSF contains two consensus RNA-binding domains and an unusual amino terminus rich in proline and glutamine residues. The RNA-binding properties of PSF are apparently identical to those of PTB. Both proteins, together and independently, bind the polypyrimidine tract of mammalian introns.⁵ However, the nuclear localization of PSF and PTB and their distribution in subnuclear fractions differ markedly: isolated nuclear matrices contain a bulk of PSF, but only minor amounts of PTB. In confocal microscopy both proteins appear in speckles, the majority of which do not co-localize. These PTB/PSF complexes, as well as the observed PSF-PTB interaction, may reflect the presence of PTB and PSF in spliceosomal complexes during RNA processing, although other data point to different cellular distribution and nuclear matrix association of the majority of PSF and PTB.⁶ The cleavage of PSF during lysis of immature myeloid cells is accompanied by digestion of the PTB splicing regulator but not other proteins tested. In contrast, during apoptosis PTB is degraded while PSF remains intact.³ Proteolytic degradation of PSF specifically occurs in intact myeloid cells and this process is enhanced upon immature myeloid cell lysis; PSF is completely cleaved to a 47 kDa proteolytic cleavage product (p47), due to potent proteolytic activity found in these cells but rare in other cells and tissues. Furthermore, p47 is abundant in intact normal and tumor myeloid cells while in other cell types it is undetectable. The bone marrow 47 kDa protein is a fragment constituting the N-terminal, protease-resistant half of the splicing factor PSF.³ PSF is highly basic and migrates anomalously on SDS gels. The 47 kDa protein of mouse cells of immature myeloid origin (bone marrow and acute myeloid leukemia) exhibits a gel migration pattern corresponding to a 49 kDa molecule.³ In other cell types such as lymphoid cells and in peripheral blood cells, PSF appears as approx. 100 kDa or 75 kDa molecules.^{3,7} The sequence of a fragment of mouse PSF was found to be remarkably similar to that of human PSF (> 98% homology).^{3,8} Also, the sequences of PSF and the human (h) 100 kDa DNA-pairing protein

(hPOMp100) reveals identity.⁷ Homologous pairing is a fundamental biological reaction implicated in various cellular processes such as DNA recombination and repair, chromosome pairing, sister chromatid cohesion and chromosome condensation, gene inactivation and initiation of replication. The base pairing is also involved in spliceosome assembly resulting in formation of a dynamic Holliday-like structure within which splicing occurs.⁷ Indeed, PSF/hPOMp100 bind both single-stranded (ss) and double-stranded (ds) DNA and facilitates the renaturation of complementary ssDNA molecules. Importantly, PSF/hPOMp100 promotes the formation of D-loops in superhelical duplex DNA. PSF/hPOMp100 also serves as an efficient substrate for protein kinase C (PKC) *in vitro*. PKC phosphorylation of PSF/hPOMp100 stimulates its DNA binding and D-loop formation activity suggesting a possible regulatory mechanism.⁷

PSF has been demonstrated to interact with a variety of cellular targets including the human pro-oncoproteins EWS, hPOMp75/TLS and calmodulin, the RNA/DNA-binding nuclear protein p54^{nrB}/NonO (the homolog of PSF) and DNA topoisomerase.^{7,9} A direct interaction has been observed, between PSF and topoisomerase I which has been implied in DNA recombination, DNA repair, and chromosome formation and may act as a transcription factor and a protein kinase.⁹

PSF is also expressed by differentiating neurons in developing mouse brain. Both the expression of PSF mRNA in cortex and cerebellum and PSF immunoreactivity in all brain areas has been found to be high during embryonic and early postnatal life. In adult tissue, only various neuronal populations in the hippocampus and olfactory bulb express PSF.⁸ PSF is expressed by differentiating neurons but not by astrocytic cells including radial glia; however oligodendrocytes differentiating *in vitro* were found to express it. The restricted expression of PSF suggests that it is involved in the control of neuronal-specific splicing events occurring at particular stages of neuronal differentiation and maturation.⁸ Monoclonal antibodies reacting specifically with PSF are useful tools for the molecular identification and characterization of the functional activity of PSF.

Reagent

Monoclonal Anti-PSF is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: Approx. 2 mg/ml.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

A working concentration of 2 to 4 µg/ml is determined by immunoblotting, using whole extract of cultured HeLa cells.

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

References

1. Lee, B.-C., et al., *Blood*, **87**, 2283-2291 (1996).
2. Carramolino, L., et al., *Gene*, **195**, 151-159 (1997).
3. Shav-Tal, Y., et al., *Exp. Hematol.*, **28**, 1029-1038 (2000).
4. Krainer, A., and Maniatis, T., in: *Frontiers in Molecular Biology: Transcription and Splicing*, Hames, B.D., and Glover, D.M. (eds.), pp. 131-296, IRL press, Oxford (1988).
5. Patton, J.G., et al., *Genes Dev.*, **7**, 393-406 (1993).
6. Meissner, M., et al., *J. Cell. Biochem.*, **76**, 559-566 (2000).
7. Akhmedov, A.T., and Lopez, B.S., *Nucleic Acids Res.*, **28**, 3022-3030 (2000).
8. Chanas-Sacre, G., et al., *J. Neurosci. Res.*, **57**, 62-73 (1999).
9. Straub, T., et al., *J. Biol. Chem.*, **273**, 26261-26264 (1998).

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