

Product Information

Monoclonal Anti-Splicing Factor SC-35, clone SC-35 produced in mouse, ascites fluid

Catalog Number **S4045**

Product Description

Monoclonal Anti-Splicing Factor SC-35 (mouse IgG1 isotype) is derived from the SC-35 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized RBF-DNJ mouse. Partially purified mammalian spliceosomes were used as the immunogen.¹ The isotype is determined using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Monoclonal Anti-Splicing Factor SC-35 recognizes a phospho-epitope of the non-snRNP (small nuclear ribonucleoprotein particles) factor SC-35.¹⁻³ It is reactive with the SC-35-related non-snRNP factor SF2/ASF.^{3,4} The antibody reacts with the splicing factor SC-35 (a doublet of 35 kD).¹⁻⁴ In immunohistology^{1-3,5-13} and immunoelectronmicroscopy,² the antibody labels SC-35 as a speckled pattern that occupies a portion of the nucleoplasm, excluding the nucleoli.^{2,5} The product may be used in ELISA¹, for inhibition and depletion of splicing activity in nuclear extracts,^{1,3,4,14} for immunoaffinity purification,^{1,2} and in immunoprecipitation.^{1,14} Cross-reactivity has been observed with human,^{1-5,8-13} rat,⁶ newt,⁷ frog⁷ and *Drosophila*.

Monoclonal Anti-Splicing Factor SC-35 may be used for the localization of SC-35 using ELISA, immunohistology and immunoelectronmicroscopy, for inhibition and depletion of splicing activity in nuclear extracts, and for immunoaffinity purification and immunoprecipitation.

The RNAs that direct protein synthesis in animal 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, known as exons, interspersed with noncoding sequences, known as introns. In a process termed splicing, introns are efficiently removed before the pre-mRNA is transported from the nucleus to the cytoplasm, where it is translated.¹⁵ Studies have shown that nuclear

pre-mRNA splicing takes place in a multi-component structure termed a spliceosome. Major subunits of spliceosomes are U1, U2, U4/U6 and U5 small nuclear ribonucleoproteins (snRNP's). In addition to the snRNP's, a number of protein factors have been identified which are required for spliceosome assembly and splicing. For example, the protein factors U2AF, SF2 and SF3 are required for the binding of the U2 snRNP to the intron branch-point and for assembly of the pre-splicing complex. Two other non-snRNP splicing factors, SF2/ASF and SC-35 (splicing component of 35 kD, also termed PR264), are both required for the first step of splicing and spliceosome assembly. SF2/ASF and SC-35 are also involved in 5N splice site selection of alternatively spliced pre-mRNA's. These serine/arginine-rich (SR) proteins are conserved from *Drosophila* to man. They have similar electrophoretic mobilities, and both contain an N-terminal ribonucleoprotein (RNP)-type RNA-recognition motif and a C-terminal arginine/serine-rich domain. However, SF2/ASF and SC-35 are encoded by different genes and display only 31% amino acid sequence identity. Splicing of different pre-mRNAs may require distinct sets of SR proteins, and the commitment by SR proteins may be a critical step at which alternative and tissue-specific splicing is regulated. The essential non-snRNP splicing factor SC-35 displays a speckled distribution in the nucleus that co-localizes with snRNPs, but unlike snRNPs, SC-35 does not give diffuse nuclear labelling.¹ In the nucleus, snRNPs are concentrated in coiled bodies and in the speckled regions, whereas SC-35 is found in speckles but not in coiled bodies.¹⁶ An understanding of the molecular organization of the mammalian cell nucleus can provide insights into the mechanisms involved in coordinating RNA transcription, processing and transport.² Studies may be greatly aided by the availability of antibodies that react specifically with individual splicing factors, such as the anti-SC-35 antibody.

Reagent

Supplied as ascites fluid with 15 mM sodium azide as a preservative.

Precautions

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

For continuous use, store at 2-8°C for up to one month. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

Indirect immunofluorescence: a dilution of at least 1:2,000 was determined by staining cultured human fibroblasts.

Note: In order to obtain best results, it is recommended that each individual user determine working dilution by titration assay.

References

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Indirect Immunofluorescence Labeling of Cultured Cells

A. Materials:

1. Cultured cells grown on coverslips or chamber/slides.
2. PBS, pH 7.2-7.4, Catalog Number P4417
3. Solution A: 0.2% Triton™ X-100, 2% formaldehyde, in PBS.
4. Acetone in staining jar, at -20°C.
5. Monoclonal Anti-Splicing Factor SC-35, Catalog Number S4045.
6. Secondary antibody: Anti-Mouse IgG-FITC, Catalog Number F5262.
7. Aqueous mounting medium.
8. Coverslips (24 X 50 mm).

B. Fixation:

1. Remove cells from incubator, discard medium.
2. Rinse with PBS; drain excess solution.
3. Fix in solution A, 10 minutes at room temperature.
4. Drain excess solution A and incubate in acetone for 5 minutes at -20°.
5. Wash three times (5 minutes each) with PBS.

C. Indirect Immunofluorescent Labeling:

6. Dilute Monoclonal Anti-Splicing Factor SC-35 in PBS to appropriate dilution. Incubate for 60 minutes at room temperature in a humidity chamber.
7. Wash as in step 5.
8. Dilute Anti-Mouse IgG-FITC to appropriate dilution in PBS. Incubate for 30 minutes at room temperature in a humidity chamber.
9. Wash as in step 5.
10. Drain excess PBS.
11. Mount and cover with coverslip.

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