

Data Sheet

Anti-U2AF⁶⁵ Antibody, Mouse Monoclonal

Clone MC3, purified from hybridoma cell culture

U4758

Product Description

Anti-U2AF⁶⁵ antibody, monoclonal (mouse IgG2b isotype) is derived from the MC3 hybridoma produced by the fusion of mouse myeloma cells (Ag8.653 cells) and splenocytes from BALB/c mice immunized with recombinant human U2AF⁶⁵.¹ The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Cat. No. ISO2.

Anti-U2AF⁶⁵ Monoclonal recognizes human,¹ rat,¹ mouse,² and *Xenopus* U2AF⁶⁵.¹ It does not recognize *Drosophila* U2AF⁶⁵. The antibody can be used in ELISA,¹ immunoblotting (approx. 65 kDa),¹ immunoprecipitation, immunohistochemistry, immunocytochemistry,^{1, 2} and immunoelectron microscopy.

The transcription of a human protein-coding gene produces an average primary sequence of 27,500 nucleotides that is called pre-mRNA. This RNA consists of long introns (an average of 3,000 nucleotides in length) and short exons (an average of 145-300 nucleotides). During the maturation of the RNA to mRNA, the introns are removed and the exons are kept. This is performed by spliceosomes, which are a complex of proteins that recognize the junctions between exons and introns (splice site) and catalyze the removal of the introns.³ There are between 100,000-200,000 spliceosomes per mammalian cell nucleus. These complexes consist of sixty different proteins and five small nuclear uridine-rich small nuclear RNAs that act as ribozymes.³

U1, U2, U4/U6, and U5 are small nuclear riboprotein (snRNPs) that are major subunits of spliceosomes. Spliceosomes also contain non snRNPs proteins such as ASF/SF2 (alternative splicing factor/splicing factor 2), SC-35 (35 kDa spliceosomal component), and U2AF (U2 snRNP auxiliary splicing factor).^{1, 2}

These three proteins contain a consensus type RNA binding domain and a region of Arg/Ser (RS) repeats. U2AF (consisting of U2AF³⁵ and U2AF⁶⁵ subunits) is a splicing factor that binds U2snRNP to the pre-mRNA branch points by the RS repeats located at the N-terminal part of the U2AF⁶⁵. The RNA binding domain of U2AF⁶⁵ binds to the polypyrimidine tract adjacent to the 3' splice site.^{1, 2} Both U2AF⁶⁵ and U2AF³⁵ shuttle continuously between the nucleus and the cytoplasm. The RS domain in both U2AF⁶⁵ and U2AF³⁵ act as a nuclear localization signal and the presence of only one of them is enough for the localization of both factors to the nucleus.^{1, 2}

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: ~ 2 mg/mL.

Precautions and Disclaimer

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

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze 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. Working dilutions should be discarded if not used within 12 hours.

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Product Profile

Immunoblotting: A working antibody concentration of 0.1-0.2 µg/mL is recommended using total cell extract from HeLa cells.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilution by titration.

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References

1. Gama-Carvalho, M., et al., J. Cell Biol., 137, 975-987 (1997).
2. Gama-Carvalho, M., et al., J. Biol. Chem., 276, 13104-13112 (2001).
3. Garcia-Blanco, M.A., et al., J. Clin. Invest., 112, 474-480 (2003).

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