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Product Information

Monoclonal Anti-Ku Antigen

Clone Ku15

Mouse Ascites Fluid

Product Number **K 2882**

Product Description

Monoclonal Anti-Ku Antigen (mouse IgG1 isotype) is derived from the Ku15 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a preparation of human epidermoid carcinoma cell line A431. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Ku Antigen recognizes an epitope included within a.a. 490-707 of the 86 kDa Ku subunit. The product is reactive in immunoblotting, immunoprecipitation, immunocytochemical staining of nuclei in cultured cells and in immunohistochemical labeling of frozen, Methacarn- and formalin-fixed, paraffin-embedded tissues. The antibody is reactive with human and monkey antigen.

A eukaryotic cell must rapidly and reversibly alter its patterns of gene expression in response to changes in its environment to function effectively. One of the most lethal lesions that can occur in a cell is a DNA double-strand break which disrupts the integrity of the DNA molecule. DNA double-strand breaks (dsbs) are introduced by x-rays and oxidative metabolism and their repair is essential to cell survival. This is achieved through modulation of the activity of sequence-specific, DNA-binding transcription factors that is often mediated by phosphorylation. This phosphorylation requires the presence of DNA and is catalyzed by the DNA-dependent protein kinase (DNA-PK).^{1,2} DNA-PK, also known as template-associated protein kinase, is a serine/threonine kinase that is absolutely dependent for activity on binding to double-stranded DNA containing broken ends, nicks and single-stranded gaps. DNA-PK phosphorylates many protein substrates *in vitro* including the 90 kDa heat-shock protein (hsp90), Sp1, SV40 T antigen, the tumor suppressor protein p53, serum response factor (SRF), fos, jun, and the C-terminal domain (CTD) of RNA polymerase II.² In this capacity, it plays a key role in initiation of transcription. DNA-PK has been purified and characterized from many human cells

and tissues and consists of at least two protein components, a large polypeptide of approximately 350 kDa (p350) and the Ku autoantigen. The p350 component binds adenosine triphosphate (ATP) analogs and contains the kinase domain of DNA-PK. The Ku protein is a heterodimer composed of two subunits, 70 kDa and 86 kDa (80 in some publications), that was originally detected as an autoantigen reacting with antibodies from patients with rheumatic disorders.³ The biochemical properties of both Ku and DNA-PK suggest that they have a role in DNA damage detection or in repair or in both processes. The localization of this protein complex to the DNase-sensitive regions in the chromatin was the first indication that the Ku protein may be involved in active DNA processes such as transcription, replication, or repair. For instance, it was reported that the Ku protein is involved in specific binding to the promoter region of human transferrin receptor gene and to the proximal and distal promoter elements of the U1 snRNA gene. The *in vitro* transcription of U1 snRNA gene depends to a large extent on the presence of the Ku protein.⁴ Ku interacts with the terminals of double-stranded DNA through the 70 kDa subunit and by this means provides the DNA targeting required by DNA-PK. Ku p86 has been identified as the XRCC5 (human DNA repair gene product), thus it is involved in DNA repair and in V(D)J recombination. Ku can complement both the radiosensitivity and the V(D)J deficient phenotypes of radiosensitive (XRS) cell lines.^{5,6} Monoclonal antibody reacting specifically with the p86 subunit of the Ku protein may be used to study of the processes involved in the genetic expression.

Monoclonal Anti-Ku Antigen may be used for the localization of the Ku antigen using various immunochemical assays including immunoblot, immunoprecipitation, immunohistochemistry, and immunocytochemistry.

Reagents

The product is provided as ascites fluid with 0.1% sodium azide as a preservative.

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

For continuous use, store at 2-8 °C. 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.

Product Profile

An antibody titer of a least 1:10,000 was determined by indirect immunofluorescent labeling of cultured human fibroblasts.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

References

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4. Zhang, W. W., and Yaneva, M., *Biochem. Biophys. Res. Commun.*, **186**, 574 (1992).
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