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Antibodies to RNA splicing factors

Anti-DEK antibodies (DEK-101AP)

Eukaryotic mRNAs exist *in vivo* as ribonucleoprotein particles (mRNPs). The protein components of mRNPs have important functions in mRNA metabolism, including effects on subcellular localization, translational efficiency and mRNA half-life. The pre-mRNA splicing can alter mRNP structure and thereby affect downstream mRNA metabolism. The spliceosome stably deposits several proteins on mRNAs as a single complex of approximately 335 kDa. This complex contains several components including the five components that make up the splicing-associated factors are SRm160, DEK and RNPS1, the mRNA-associated shuttling protein Y14 and the mRNA export factor REF (1). Human Daxx (360 kDa) is a protein that functions, in part, as a transcriptional co-repressor through its interaction with a growing number of nuclear, DNA-associated proteins including DEK, a chromatin-associated protein reported to change the topology of DNA in chromatin *in vitro* and negatively regulate the transcriptional activity.

Discrimination between splice sites and similar, non-splice sequences is essential for correct intron removal and messenger RNA formation in eukaryotes. DEK, a chromatin- and RNA-associated protein mutated or over-expressed in certain cancers, enforces 3' splice site discrimination by 35 and 65 kDa subunits of U2AF. DEK phosphorylated at serines 19 and 32 associates with U2AF35, facilitates the U2AF35-AG interaction and prevents binding of U2AF65 to pyrimidine tracts not followed by AG. DEK and its phosphorylation are required for intron removal, but not for splicing complex assembly, which indicates that proofreading of early 3' splice site recognition influences catalytic activation of the spliceosome (3). The DEK proto-oncogene has been associated with human carcinogenesis—either as a fusion with the CAN nucleoporin protein or when transcriptionally upregulated. Mechanisms of intracellular DEK functions, however, have remained relatively unexplored. The chromatin-associated protein DEK was first identified as a fusion protein in patients with a subtype of acute myelogenous leukemia and has been seen associated with a number of other ailments including cancer and autoimmune diseases. The C-terminal region in the Dek (309-375) can reverse the characteristic abnormal DNA-mutagen sensitivity in fibroblast form ataxia-telangiectasia (A-T) patients (2). Dek plays a critical role in cell survival; over-expression of DEK resulted in significant life span extension of primary human keratinocytes. DEK expression protects cancer and primary human cells from apoptotic cell death. Cell death in response to DEK depletion was accompanied by increased protein stability and transcriptional activity of the p53 tumor suppressor and consequent up-regulation of known p53 target genes such as p21CIP and Bax (4).

The Dek-selective antibodies were generated against peptide from the C-terminal end of protein. The antibodies to Dek are affinity purified over immobilized antigen based chromatography, and the purified immunoglobulins are stabilized in antibody stabilization buffer. FabGennix Int. Inc., will also provide limited quantities of antigenic blocking peptides for Dek antibodies. FabGennix International Inc. also carries antibodies to proteins targets involved in transcription and RNA splicing, for a complete listing please visit www.fabgennix.com. *FabGennix Inc.* will also conjugate antibodies with fluorescent probes upon request at a reasonable cost.

Catalog #	Host Species	Nature	Cross reactivity	Quantity	price
DEK-101AP	Rabbit	Affinity purified DEK antibody	H, M, R, cat, mon	100 ug	235
P-DEK	n/a	Antigenic blocking peptide for DEK-101AP	n/a	250 ug	115
PC-DEK	n/a	Western blot positive control for DEK	n/a	5 appl.	185

R = rat; M = mouse; H = human; C = chicken; monk = monkey ; * not all variants are labeled equally

Immunogen: Synthetic peptides corresponding to positions: DEK-101AP (347-364), the peptide sequence (ckk vye nyp tyd lte rkd) unique to DEK protein.

Concentration: DEK-101AP: IgG concentration 0.65-0.85 mg/ml in antibody stabilization buffer.

Applications: Antibody DEK-101AP is ideal for WB applications for detection of human, rat, mouse or monkey DEK protein. These antibodies do not cross react to other RNA splicing factors from mammals or to other cellular proteins. The species cross reactivity for this antibody has not been examined. The dilutions for this antibody is for reference only, investigators are expected to determine the optimal conditions for specific assay. WB: > 1:500-750; IMM & i.p pull-down assays: n.d; IHC n.d.

Reactivity: This antibody detects a single band of approximately 48-50 kDa in DEK Western blot positive control (Cat # PC-DEK) samples. The antibody does not cross reacts with other proteins.

Protocols: Standard protocol for various applications (WB; IMM and IHC) of this antibody is provided with the product specification sheet, however, FabGennix Inc. strongly recommends investigators to optimize conditions for use of this antibody in their laboratories.

Form/Storage: The antiserum is supplied in antibody stabilization buffer. The affinity-purified antibodies are isolated on immobilized antigen-affinity column and supplied as stabilized product. Store at -20°C for long-term storage. FabGennix Inc. does not recommend storage of very dilute antibody solutions unless they are prepared in specially formulated multi use antibody dilution buffer (Cat # DiluOBuffer). Working solutions of antibodies in DiluOBuffer should be filtered through 0.45µm filter after every use for long-term storage.

Notes: Now Western blots can easily be stripped and recycle using our specially formulated StripOBuffer (Cat # FGI-1989). This stripping buffer does not require heating or have any pungent smell. The membranes (Nitrocellulose, Immobilon, supported nitrocellulose etc.) can be stripped and re-probed up to 10 times with any loss in enhanced chemiluminescence's signal.

References:

- Le Hir H, Izaurralde E, Maquat LE, Moore MJ. The spliceosome deposits multiple proteins 20-24 nucleotides upstream of mRNA exon-exon junctions. *EMBO J.* 2000 Dec 15;19(24):6860-9.
- Devany M, Kotharu NP, Matsuo H. Solution NMR structure of the C-terminal domain of the human protein DEK. *Protein Sci.* 2004 Aug;13(8):2252-9. Epub 2004 Jul 6.
- Soares LM, Zanier K, Mackereth C, Sattler M, Valcarcel J. Intron removal requires proofreading of U2AF/3' splice site recognition by DEK. *Science.* 2006 Jun 30;312(5782):1961-5. Related Articles, Links
- Wise-Draper TM, Allen HV, Jones EE, Habash KB, Matsuo H, Wells SI. Apoptosis inhibition by the human DEK oncoprotein involves interference with p53 functions. *Cell Biol.* 2006 Oct;26(20):7506-19. Epub 2006 Aug 7.

For users who may require large amounts of DEK-101AP, please enquire about bulk material discounts.
This Product is for Research Use Only and is NOT intended for use in humans or clinical diagnosis.

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