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ProductInformation

MONOCLONAL ANTI-E2F1,
CLONE KH20
Purified Mouse Immunoglobulin

Product Number **E 8901**

Product Description

Monoclonal Anti-E2F1 (mouse IgG2a isotype) is derived from the KH20 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a recombinant human E2F1 protein.[1] 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). The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-E2F1 reacts specifically with E2F1, and does not detect the other members of the E2F family. The epitope recognized by the antibody resides in the N-terminal region of human E2F1 (a.a. 1-89).[1] The antibody may be used for immunoprecipitation[1,2] (native and denatured), immunoblotting[3-5] (53 kDa, for natural molecule; may vary in a recombinant preparation), immunocytochemistry,[5] and gel mobility shift assay.[1,2,6] Cross-reactivity has been observed with human,[1-6] rat, mouse and *Xenopus* E2F1.

Before any gene can be translated into protein, it must first be transcribed in the nucleus into messenger RNA (mRNA), which stores a complementary copy of the DNA code. The initial event in this process is the binding of specific proteins to the enhancer and the promoter. The binding of these proteins depends on their recognition of specific nucleotide sequences. Whereas some DNA-binding proteins are "positive regulators" that stimulate transcription, others are "negative regulators" that block transcription. E2Fs transcription factors are DNA-binding proteins belonging to a family of proteins that bind to the sequence TTTCGCGC, and regulate the expression of various cellular and viral promoters.[7] These proteins associate with negative regulators, such as the retinoblastoma (Rb) proteins p107, p110 (also designated Rb, or pRb) and p130, resulting in an altered rate of gene transcription. The retinoblastoma proteins, referred to collectively as pocket proteins, constitute a nuclear protein family that share a common structural unit (the pocket) dedicated to binding certain proteins, such as certain members of the E2F family.[8-10] Generally, when an E2F species interacts with a pocket protein its ability to activate certain genes is suppressed, and the pocket protein/E2F complex acquires transrepression function.[11] All known pocket proteins bind certain viral oncoproteins, i.e., papovirus T antigen, adenovirus E1A, and human Papilloma virus E7.[9,10] Six different E2F species (E2F1-E2F6) and two DP members (DP1 and DP2) have been identified and characterized. E2Fs and DPs (dimerization partners) form heterodimers, and the "active" E2F transcriptional complexes bind to and regulate the transcription of several genes involved in the control of the cell cycle regulation and of DNA replication.[8,12] One of them, E2F5, also plays a unique role in the behavioral control of a postmitotic cell, the cerebrospinal fluid secretory cell of the choroid plexus.[13] Each of the E2F proteins, E2F1 (~53 kDa), E2F2 (~53 kDa), E2F3 (~57 kDa) and E2F4 (~50 kDa), are regulated by complex formation with Rb p110, while the p107 and p130 proteins seem to exhibit a specificity for particular E2F family members. The expression of E2F1, E2F2, and E2F3 genes is very tightly coupled to cell growth. Little or no expression of these genes is seen in quiescent cells, whereas transcription of each gene is rapidly induced after growth stimulation. In contrast, the expression of E2F4 and E2F5 genes seems to be relatively constant in relation to cell growth, exhibiting only modest increases as cells are stimulated to grow. E2F1 transcription activity is closely regulated during the cell cycle. In G_1, it is under Rb control, and Rb/E2F1-DP complexes can induce a state of G_1 arrest.[8] In late G1, cyclin-dependent kinases phosphorylate Rb, leading to the dissociation of E2F1/DP heterodimers from Rb with the reappearance of their transactivation function.

Indeed, overall E2F transactivation activity peaks at G_1/S and early S and decreases in late S.[14] It seems that in late S, cyclin A/ Cdk2 binds to E2F1 (as well as to E2F2 and E2F3) and phosphorylates the relevant DP partner, thereby suppressing E2F DNA binding activity.[15] Another level of regulation of E2F1 function is reflected by its cell cycle-dependent synthesis. E2F1 gene expression is barely detectable in G_0. By contrast, it rises as cells exit from G_0, approaching late G_1. One or more E2F species activate(s) the E2F1 promoter during the G_0 exit process, resulting in increase in E2F1 RNA and protein synthesis. Conversely, transcription of the E2F1 gene decreases in late S, possibly because of negative regulation of the DNA binding activity of E2F1-3 by cyclin A/Cdk2.[16] Antibodies reacting specifically with E2F2 are useful tools in the study of the detailed mechanisms of the control of transcription in intracellular pathways, and its essential roles during developmental and pathological processes.

Reagents
Monoclonal Anti-E2F1 is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4 containing 1% BSA and 15 mM sodium azide.

Antibody Concentration: Approximately 0.5 mg/ml

Precautions and Disclaimer
Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution.
Consult the MSDS for information regarding hazardous 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 at –20°C. 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 1-2 µg/ml is determined by immunoblotting using whole extract of transfected 293T (human embryonal kidney) cells expressing E2F1.
Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

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lpg 7/00