



3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sia.com
sigma-aldrich.com

Product Information

MONOCLONAL ANTI-DP2

CLONE BC2

Purified Mouse Immunoglobulin

Product Number **D 7438**

Product Description

Monoclonal Anti-DP2 (mouse IgG1 isotype) is derived from the BC2 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a recombinant human DP2 protein. The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2). The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-DP2 reacts specifically with DP2 and does not detect the other members of the DP family. The antibody may be used in immunoprecipitation (native and denatured), immunoblotting (47 kDa) and gel mobility shift assay. Reactivity has been observed with human DP2.

Before any gene can be expressed by translation into amino acids it must first be transcribed in the nucleus into messenger RNA (mRNA) that 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. While some DNA-binding proteins are "positive regulators" that stimulate transcription, others are "negative regulators" that block transcription.

E2Fs (E2 factors) are DNA-binding proteins (transcription factors) consisting of a family of proteins that bind to the sequence TTTCGCGC and regulate the expression of various cellular and viral promoters.¹ 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.²⁻⁴ 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.⁵ All known pocket proteins bind certain viral oncoproteins, i.e., papovirus T antigen, adenovirus E1A, and Human Papilloma virus E7.^{3,4}

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 their "active" E2F transcriptional complexes bind to and regulate the transcription of several genes involved in control of the cell cycle regulation and DNA replication.^{2,6} Each of the E2F proteins is 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 DP2 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 G₁, it is under Rb control, and Rb/E2F1-DP complexes can induce a state of G₁ arrest.^{2,7} 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 G₁/S and early S and decreases in late S.⁸ 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.⁹ Antibodies reacting specifically with DP2 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-DP2 is supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide (see MSDS) as a preservative.

Antibody Concentration: Approx. 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. 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 0.5 - 1 µg/ml is determined by immunoblotting using a whole extract of transfected 293T (human embryonal kidney) cells expressing DP2.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

1. Zhang, Y., and Chellappan, S.P., *Oncogene*, **10**, 2085-2093 (1995).
2. Nevins, J.R., *Cell Growth Differ.*, **9**, 585-593 (1998).
3. Sellers, W.R., and Kaelin, W.J., *J. Clin. Oncol.*, **15**, 3301-3312 (1997).
4. Weinberg, R.A., *Cell*, **81**, 323-330 (1995).
5. Weintraub, S.J. et al., *Nature*, **375**, 812-815 (1995).
6. Dyson, N., *Genes Dev.*, **12**, 2245-2262 (1998).
7. Martelli, F., and Livingston, D.M., *Proc. Natl. Acad. Sci. USA*, **96**, 2858-2863 (1999).
8. Wade, M., et al., *Mol. Cell Biol.*, **12**, 4364-4374 (1992).
9. Leone, G., et al., *Genes Dev.*, **12**, 2120-2130 (1998).

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.