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ProductInformation

Monoclonal Anti-Granzyme B Clone GrB7 Mouse Culture Supernatant

Product Number G 1044

Product Description

Monoclonal Anti-Granzyme B (mouse IgG2a isotype) is derived from the GrB7 hybridoma produced by the fusion SP2/O mouse myeloma cells and lymph nodes cells from a BALB/c immunized with a recombinant human granzyme B. The antibody is concentrated from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-Granzyme B recognizes the 33 kDa human serine protease granzyme B. The antibody does not cross-react with granzyme A. The antibody may be used in immunohistochemistry (pretreated formalin-fixed, paraffin-embedded sections) and immunoblotting. The antibody performs poorly on frozen sections.

Cytotoxic granules secreted by natural killer (NK) cells and cytotoxic T lymphocytes are part of the mechanism used for protecting the organism from virus infection and tumor cells. Granzyme B, a serine protease, is the most prominent granzyme in a family of eleven all found in cytotoxic granules.¹⁻⁵ The granzymes enter the target cell with the assistance of perforin, a critical molecule of the cytotoxic granules. In the target cell, the granzymes act on specific substrates involved with the cell death via apoptosis. Granzyme B, a 247 amino acid polypeptide, contains a leader sequence (cleaved by a signal peptidase) and two amino acid prodomains (cleaved by the lysosomal cysteine protease DPPI).⁶ Granzyme B is a neutral serine protease that cleaves aspartic acid residues, inducing cell death by various pathways.7-9 It can cleave and activate most of the caspases in vitro and in vivo resulting in a massive amplification of the caspase dependent apoptotic pathway. In addition, granzyme B cleaves directly downstream caspase substrates as PARP DNA-PK2 and DFF45/ICAD¹⁰ leading to cell death. This pathway bypasses inhibition of apoptosis by viral caspase inhibitors found in virus-infected cells. It has been shown that granzyme B is capable of inducing cytochrome C release from the mitochondria in a caspase independent way.¹¹

Reagent

Monoclonal Anti-Granzyme B is supplied as a solution in serum-free culture medium, containing 0.7% bovine serum albumin and 0.1% sodium azide.

Antibody concentration: Approx. 250 μ g/ml **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 up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is also 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.

Product Profile

A working dilution of 1:20 is determined using immunohistochemistry on granzyme B expressing lymphocytes in sections of sublimate formaldehyde as well as formalin-fixed, paraffin-embedded tissues. Before staining, the slides must be pretreated with 0.1 M sodium citrate, for 10 minutes at 100 °C (in microwave oven).

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

References

- Kummer, J.A., et al., Production and characterization of monoclonal antibodies raised against recombinant human granzymes A and B and showing cross reactions with the natural proteins. J. Immunol. Methods, **163**, 77-83 (1993).
- Kummer, J.A., et al., Localization and identification of granzymes A- and B-expressing cells in normal human lymphoid tissue and peripheral blood. Clin. Exp. Immunol., **100**, 164-172 (1995).
- de Bruin, P.C., et al., Granzyme B-expressing peripheral T-cell lymphomas: neoplastic equivalents of activated cytotoxic T cells with preference for mucosa-associated lymphoid tissue localization. Blood, 84, 3785-3791 (1994).
- Oudejans, J.J., et al., Granzyme B expression in Reed-Sternberg cells of Hodgkin's disease. Am. J. Pathology, **148**, 233-240 (1996).

- Oudejans, J.J., et al., Analysis of major histocompatibility complex class I expression on Reed-Sternberg cells in relation to the cytotoxic T-cell response in Epstein-Barr virus-positive and -negative Hodgkin's disease. Blood, 87, 3844-3851 (1996).
- Smyth, M.J., et al., J. Immunol., 154, 6299-6305 (1995).
- Shresta, S., et al., Curr. Opin. Immunol., 10, 581-587 (1998)
- 8. Trapani, J.A., et al., Immunology Today, **20**, 351-356, 1999.
- 9. Trapani, J.A., et al., Curr. Opin. Immunol., **12**, 323-329 (2000).
- 10. Thomas, D.A., et al., Immunity, **12**, 621-632 (2000).
- 11. Heibein, J.A,. et al., J. Immunol., **163**, 4663-4693 (1999).

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