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Product Information

Anti-Cytochrome c
produced in sheep, affinity isolated antibody

Catalog Number **C9616**

Product Description

Anti-Cytochrome c is produced in sheep using purified rabbit cytochrome c conjugated to KLH as immunogen. The antibody is affinity-purified using the immunizing protein immobilized on agarose.

Anti-Cytochrome c recognizes human, rat, rabbit, and dog cytochrome c. Applications include immunoblotting (15 kDa), immunocytochemistry, and immunohistochemistry. Detection of the cytochrome c band by immunoblotting is specifically inhibited with the immunizing protein.

A prominent role for mitochondria in controlling cell death has emerged in recent years. Mitochondrial cytochrome c, a nuclear DNA encoded protein, has been found to have dual functions in controlling both cellular electron transport and energy metabolism[1] as well as apoptosis.[2] Apocytochrome c, its precursor, is synthesized on free ribosomes in the cytoplasm and can spontaneously insert into the mitochondrial outer membrane via a non-receptor mediated process.[3, 4] With its further interaction with mitochrondrial cytochrome c heme lyase, heme is incorporated, and the protein refolds and is released into the mitochondrial intermembrane space. The functional cytochrome c then binds with cytochrome oxidase via its surface positive charges.

As part of the mitochondrial electron transport chain, cytochrome c has a very well defined and specific function in transfer of electrons between complex III (ubiquinol: cytochrome c oxidase) and complex IV (cytochrome oxidase). Cytochrome c is an essential component of the complex that activates the death protease caspase-3 (CCP32). During apoptosis, cytochrome c is released from mitochondria and this is inhibited by the presence of Bcl-2 on the organelles.[5, 6]

Cytosolic cytochrome c forms an essential part of the vertebrate ”apoptosome”, which is composed of cytochrome c, Apaf-1, and procaspase-9.[7] The result is activation of caspase-9, which then processes and activates other caspases to direct apoptosis. In cells induced by several apoptotic agents, (such as UV irradiation, staurosporine, and overexpression of Bax), caspase inhibitors do not prevent cytochrome c release.[8-10] However, an exception is found with the Fas pathway.[9, 11]

The model is emerging that once cytochrome c is released, the cell is committed to die by either a rapid apoptotic mechanism involving Apaf-1 mediated caspase activation or a slower necrotic process due to collapse of electron transport, which occurs when cytochrome c is depleted from mitochondria. Cytochrome c is a highly conserved protein and cytochrome c from horse, bovine, rat, pigeon, and tuna all could reconstitute the caspase activation *in vitro*.[2, 12]

It has been reported that serum cytochrome c is a sensitive apoptotic marker *in vivo*, and increased serum levels can serve as a negative prognostic marker.[13]

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as preservative.

Antibody concentration: ~0.5 mg/mL

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards 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, or storage in “frost-free” freezers, is 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

Immunoblotting: a working concentration of 0.1-0.2 µg/mL is recommended using whole extracts of MCF-7, Jurkat, Rat-1, and MDCK cells, or rat kidney or rat heart extract, and a chemiluminescence detection reagent.

Recommendation: For optimal results, we recommend pre-blocking the membrane with 5% normal serum from the same host species of the labeled secondary antibody. The same solution is also recommended for diluting the anti-Cytochrome c antibody and the secondary antibody.

Indirect immunofluorescence: a working concentration of 5-10 µg/mL is recommended using human MCF-7 cells.

Immunohistochemistry: a working concentration of 20-40 µg/mL is recommended using formalin-fixed, paraffin-embedded sections of human heart and an indirect immunoperoxidase staining procedure.

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

References

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