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Product Information

Anti-Cytochrome c
produced in sheep, whole antiserum

Catalog Number **C5723**

Product Description

Anti-Cytochrome c (cyt *c*) is produced in sheep using purified rabbit cytochrome C as immunogen.

Anti-Cytochrome c reacts specifically with cytochrome c (12.3 kDa). Species cross-reactivities include human, rat, rabbit, and dog.

Anti-Cytochrome c may be used for the detection of Cytochrome c by immunoblotting, immunocyto-chemistry, and immunoprecipitation.

A prominent role for mitochondria in controlling cell death has recently emerged. Mitochondrial cytochrome c, a 14.4 kDa nuclear DNA encoded protein, has been found to have dual functions in controlling both cellular electron transport and energy metabolism[1] and 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 these 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 over expression 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-1mediated 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]

Reagent

The product is supplied as whole antiserum containing no carrier or preservative.

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

Store at –20 °C in working aliquots. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. For short-term use, store at 2-8 °C, for up to one month.

Procedure

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1μg/μL total cell protein in a microcentrifuge tube with lysis buffer.
2. Add 1 μL of Anti-Cytochrome c to 0.5 mg of the cell lysate.
3. Gently rock the reaction mixture at 4 °C from 4 hours to overnight.

4. Capture the immunocomplex by adding 5 µL of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (2.5 µL packed beads), Catalog Number P2545.
   Note: For greater than 3 µL of antiserum, add more beads in a ratio of 5 µL beads for every 2 µL of antiserum. Gently rock reaction mixture at 4 °C for 2 hours.
5. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x *g*), and drain off the supernatant. Add 1 ml lysis buffer and rock at 4 °C for 5 min.
6. Wash the beads 3 times with ice cold cell lysis buffer.
7. Wash the beads 2 times with 0.1 M Tris, pH 8.0, containing 0.1 M NaCl.
8. Resuspend the agarose beads in 40 µL 2x Laemmli sample buffer. The agarose beads can be frozen for later use. For a total protein lane add 1 µL lysate to 39 µL 2x Laemmli sample buffer.
9. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. The beads are pelleted by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant. Use 10 µL of each sample for a tricine gel.

Lysis Buffer:
0.1 M Tris-HCl, pH 8.0, containing 10 mM EDTA, 1 mM PMSF, and 1% Triton™ X-100.

Product Profile
Immunoblotting: working dilution is 1:5000 using Mcf-7, Rat-1, MDCK and Jurkat cell lysates, anti-sheep IgG conjugated to peroxidase and enhanced chemiluminescence.

Immunoprecipitation: suitable

Immunofluorescence: may be used in immunofluorescence applications with human and rat cells.

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

References

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