

Product Information

ANTI-CYTOCHROME C

Developed in Sheep, Whole Antiserum

Product Number **C 5723**

Product Description

Anti-Cytochrome C (cyt *c*) is developed 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, immunocytochemistry, and immunoprecipitation.

A prominent role for mitochondria in controlling cell death has recently emerged. Mitochondrial cytochrome C, a 12.3 kDa nuclear DNA encoded protein, has been found to have dual functions in controlling both cellular electron transport and energy metabolism¹ 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 mitochondrial 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-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}

Reagents

The product is supplied as whole antiserum containing 0.08% sodium azide.

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

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 $2-8^{\circ}\text{C}$, for up to one month.

Procedure

Immunoprecipitation

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly $1\mu\text{g}/\mu\text{L}$ total cell protein in a microcentrifuge tube with lysis buffer.
2. Add $1\mu\text{L}$ 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 $5\mu\text{L}$ of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads ($2.5\mu\text{L}$ packed beads) (Product No. P 2545).

Note: For greater than 3 μ l of antiserum, add more beads in a ratio of 5 μ l beads for every 2 μ l 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 μ l 2x Laemmli sample buffer. The agarose beads can be frozen for later use. For a total protein lane add 1 μ l lysate to 39 μ l 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 μ l 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.

Immunocytochemistry

1. Using sterile conditions, place 3 coverslips in one tissue culture dish.
2. Add 10 ml culture media
3. After splitting cells, add the appropriate amount of cells to each dish. Incubate 24 hours in a 37 °C. CO₂ incubator.

Note: the number of cells added to each dish depends on the individual cell growth rate. At the time of fixation, cells should be 40-50% confluent.

4. Draw off culture media and rinse the cells 2 X with warm PBS.
5. Transfer coverslips to 6 well culture dishes, one coverslip per well. It is recommended to line the bottom of the wells with parafilm for easier handling.
6. Add 30 μ l fixative (4% paraformaldehyde) for 30 min. at room temperature.
7. Wash the coverslips with PBS, 1 X for 5 min. with gentle agitation. Do not shake cells.
8. Add 30 μ l PBS containing 1% Triton-X-100 and incubate 10 min. at room temperature.
9. Wash cells with PBS containing 0.02% Tween 20 for 5 min.
10. Wash cells with PBS containing 0.02% Tween 20 and 1% BSA for 5 min.

11. Incubate the coverslips with 30 μ l anti-Cytochrome C diluted approximately 1:650 (1.5 μ l/ml) in PBS containing 3% BSA and incubate in a humidified chamber 45 min. 37 °C.
12. Wash the cells with PBS containing 0.02% Tween 20 and 1% BSA for 5 min.
13. Add 30 μ l of a 1:40 dilution of anti-sheep IgG conjugated with FITC (Product No. F 7634) in PBS containing 3% BSA and incubate in a humidified chamber 45 min. 37 °C.
14. Wash the cells with PBS containing 0.02% Tween 20 and 1% BSA for 5 min.
15. Wash the cells with PBS for 5 min.
16. Dry in a 37 °C oven (bacterial incubator) for 45 min.
17. Use 10-15 μ l mounting media (Product No. 1000-4) to mount each coverslip onto a slide. Add mounting media on side, and add coverslip with cells facing mounting media on top. Seal with clear nail polish and label slides.
18. Examine the cells under a fluorescent microscope.

Product Profile

Working dilution is 1:10,000 (0.01 μ l/ml) by immunoblotting using a Mcf-7, Rat-1, MDCK and Jurkat cell lysates, anti-sheep IgG conjugated to peroxidase and enhanced chemiluminescence.

By immunoprecipitation, 1 μ l will immunoprecipitate cytochrome C from a lysate of Mcf-7 cells.

By Immunocytochemistry, a dilution of approximately 1:650 (1.5 μ l/ml) can be used to visualize cytochrome C in a strain of Mcf-7 cells that expressing Bcl-2 using immunofluorescence.

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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