

Product Specification Sheet

**Cytochrome-C (Cyt-C) Antibodies**

Cat. # CYTC11-S	Sheep Anti-rabbit Cyt-C antiserum # 1	<b>SIZE:</b> 100 ul
Cat. # CYTC11-C	Rat Cyt-C purified protein for WB +ve control	<b>SIZE:</b> 100 ul

Cytochrome-C enzyme is a nuclear encoded protein located in the mitochondria of all aerobic cells. It is involved in the electron transport system that functions in oxidative phosphorylation. It accepts electrons from cytochrome b and transfers them to cytochrome oxidase. In the process the iron of the heme group (which is identical to that of hemoglobin and myoglobin) shifts from the ferrous to the ferric state. Human cytochrome-c has 105-aa (~12 kDa).

Mitochondria play a pivotal role in the regulation of programmed cell death or apoptosis. Apoptosis is driven by two classes of specialized proteases known as caspases (Cysteine aspartase). The initiator caspases can be activated by self-cleavage. The effector caspases are then activated in an amplification cascade. Several key participants are released from the mitochondria that regulate apoptosis. The first such factor (Cytochrome-C) to be described binds to a cytoplasmic scaffolding protein called Apaf-1. Binding of the mitochondria factor allows Apaf-1 to form a ternary complex with, and activate, the initiator pro-caspase-9. Active caspase-9 then turns on downstream effector caspases, initiating apoptosis. Murine embryos devoid of cytochrome c died in utero by midgestation. In contrast, cells lacking cytochrome c demonstrated increased sensitivity to cell death signals triggered by TNF.

**Source of Antigen and Antibodies**

<b>Antigen</b>	Rabbit Cyt-C protein
<b>Ab Host/type</b>	Sheep, Polyclonal Unpurified antiserum (cat # CYTC11-S)
<b>2-ab</b>	<b>Rabbit Anti-Sheep IgG-HRP</b> cat # 50320 (AP, biotin, FITC conj also available)
<b>-ve control IgG</b>	Cat # 20006-1, sheep (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as -ve control

Purified rat Cyt-c protein for **western blot +ve control (Cat # CYTC11-C)**, is supplied in SDS-PAGE sample buffer (reduced). Load 10 ul/lane of **CYTC11-C** for good visibility with antibody Cat # **CYTC11-S**. Store at -20oC in suitable size aliquots. SDS may crystallize in cold conditions. It should redissolve by warming before taking it from the stock. It should be heated once prior to loading on gels. If the product has been stored for several weeks, then it may be preferable to add 5 ul of fresh 2x sample buffer per 10 ul of the **CYTC11-C** solution prior to heating and loading on gels. This preparation is not biologically active. It is not suitable for ELISA or other applications where native protein is required. Do not freeze, thaw, or heat repeatedly

**Form & Storage of Antibodies/Peptide Control**

<b>Antiserum (unpurified, undiluted)</b>	
100 ul/vial solution	50 ul/vial lyophilized powder

contains 0.05% sodium azide  
**Reconstitute powder** 100 ul PBS

**Storage**

**Short-term:** unopened, undiluted vials for less than a week at 4oC.

**Long-term:** at -20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

**Stability:** 6-12 months at -20oC or below.

**Shipping:** 4oC for solutions and room temp for powder.

**Recommended Usage**

**Western Blotting** (1:1K-5K for antiserum)

**ELISA:** Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

**Histochemistry & Immunofluorescence:** We recommend a dilution 1:250-1:1000 and paraformaldehyde fixed sections of tissues.

**Immunoprecipitation:** Use 1-2 ul for cell lysate (100-200 ug protein).

Recommended dilution and concn of antibody must be optimized for all techniques.

**Specificity & Cross-reactivity**

Anti-rabbit Cyt-C reacts with mouse, rat, human, rabbit, and dog Cyt-C. Antibody cross-reactivity in various other species has not been studied. Rat cytochrome-C protein (#CYTC11-C) can be used for western as positive control.

**General References:** (1) Matubara H et al (1962) JBC 237, 3575-3576; Evans MJ et al (1988) PNAS 85, 9625-9629; Skulachev VP et al (1998) FEBS Lett. 423, 275-280; Nijhawan D et al (1997) Cell 91, 479-489; Liu X et al (1996) Cell 86, 147-157

\*This product is for In vitro research use only.

**Related material available from ADI**

Antibodies AIF, Apaf-1, Cytochrome-C, Caspases, IAPs, Survivin, EPR-1, CARD, and other Apoptosis related proteins

Pre-made BrainBlot (study distribution of proteins in 12-distinct regions of rat/mouse brain)

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Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, S an Antonio, T X 7 8 24 4 , U S A;

India Contact:

**Life Technologies (India) Pvt. Ltd.**

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi - 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400 Fax: +91-11-42208444 Email: [customerservice@lifetechindia.com](mailto:customerservice@lifetechindia.com) Website: [www.lifetechindia.com](http://www.lifetechindia.com)