# Cytochrome c Release Assay Kit ab65311

**Overview**

<table>
<thead>
<tr>
<th>Product name</th>
<th>Cytochrome c Release Assay Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample type</td>
<td>Tissue, Adherent cells, Suspension cells</td>
</tr>
<tr>
<td>Assay type</td>
<td>Direct</td>
</tr>
<tr>
<td>Assay time</td>
<td>3h 00m</td>
</tr>
<tr>
<td>Species reactivity</td>
<td>Reacts with: Mouse, Rat, Human</td>
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**Product overview**

Cytochrome c Release Assay Kit ab65311 provides an effective means for detecting cytochrome c translocation from mitochondria into cytosol during apoptosis.

The kit provides reagents to isolate a highly enriched mitochondria fraction from cytosol. The procedure is simple and easy to perform; no ultracentrifugation is required and no toxic chemicals are involved.

Cytochrome c release from mitochondria into the cytosol is determined by Western blotting using the cytochrome c antibody provided in the kit.

The anti-Cytochrome c antibody is a mouse monoclonal antibody that reacts with denatured human, mouse, and rat cytochrome c.

Cytochrome c release assay protocol summary:
- collect cells, centrifuge and wash with PBS
- resuspend in cytosol extraction buffer mix
- homogenize cells with a dounce tissue grinder
- centrifuge homogenate at 700 x g for 10 min
- collect supernatant and centrifuge at 10,000 g for 30 min, collect supernatant as cytosolic fraction
- resuspend pellet in mitochondrial extraction buffer mix and save as mitochondrial fraction
- analyze cytosolic and mitochondrial fractions in western blotting with cytochrome c antibody

**Notes**

This kit was previously called Cytochrome c Releasing Apoptosis Assay Kit.

Cytochrome c plays an important role in apoptosis. The protein is located in the space between the inner and outer mitochondrial membranes. An apoptotic stimulus triggers the release of cytochrome c from the mitochondria into cytosol where it binds to Apaf-1. The cytochrome c/Apaf-1 complex activates caspase-9, which then activates caspase-3 and other downstream caspases.
**Other apoptosis assays**

For more apoptosis assays, review the apoptosis assay and apoptosis marker guide.

**Properties**

**Storage instructions**
Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>Identifier</th>
<th>100 tests</th>
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<tbody>
<tr>
<td>5X Cytosol Extraction Buffer</td>
<td></td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>Anti-Cytochrome c Mouse mAb</td>
<td>Green</td>
<td>1 x 500µl</td>
</tr>
<tr>
<td>DTT</td>
<td></td>
<td>1 x 110µl</td>
</tr>
<tr>
<td>Mitochondria Extraction Buffer</td>
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<td>1 x 10ml</td>
</tr>
<tr>
<td>Protease Inhibitor Cocktail</td>
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<td>1 vial</td>
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</table>

**Relevance**
Cytochrome c plays an important role in apoptosis. The protein is located in the space between the inner and outer mitochondrial membranes. An apoptotic stimulus triggers the release of cytochrome c from the mitochondria into cytosol where it binds to Apaf-1. The cytochrome c/Apaf-1 complex activates caspase-9, which then activates caspase-3 and other downstream caspases.

**Cellular localization**
Mitochondrial

**Images**

Cytochrome C release was measured using Cytochrome C releasing apoptosis assay kit (ab65311). Blots showing immunoreactive bands for cytochrome c in cytosol (Image A). Data was expressed in fold-increase of cytochrome c compared to vehicle. Protein expression levels were normalized to β-actin (Figure B). Blots (Image C) of immunoreactive bands for cytochrome C in mitochondria. Figure D shows a fold-increase of cytochrome C compared to vehicle. Protein expression levels were normalized to COX IV.
5x10^7 Jurkat cells were cultured in the absence (1-2) or presence of 2 μM Camptothecin (CPT) (ab120115) for 24 hours (3-4) or with 10 μM CPT for 4 hours (5-6). 30 μL cytosolic (1, 3, 5) and mitochondrial (2, 4, 6) extracts were loaded onto the gel. Membranes were probed with anti-Cytochrome C Mouse MAb (ab65311) (dilution 1:200) followed by Goat Anti-Mouse IgG (HRP) (ab97040) (dilution 1:2000).

Bands were detected at the predicted size of 12 kDa.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"