abcam

Product datasheet

Human Cytochrome C ELISA Kit ab221832

SimpleStep ELISA®

1 References 7 Images

Overview

Product name Human Cytochrome C ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
Extract	8			3.4%

Inter-assay

Sample	n	Mean	SD	CV%
Extract	3			3.8%

Sample type Cell culture extracts, Tissue Extracts

Assay type Sandwich (quantitative)

Sensitivity 1100 pg/ml

Range 1170 pg/ml - 75000 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture extracts	107	105% - 110%
Tissue Extracts	104	101% - 106%

Assay time 1h 30m

Assay duration One step assay

Species reactivity **Reacts with:** Human

Product overview Cytochrome C *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Cytochrome C protein in human cell and tissue extracts and subcellular fractions.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Notes Cytochrome C is 11 kDa mitochondrial intermembrane space electron carrier protein. The oxidized form of the cytochrome C heme group can accept an electron from the heme group of the cytochrome c1 subunit of cytochrome reductase. Cytochrome C then transfers this electron to the cytochrome oxidase complex, the final protein carrier in the mitochondrial electron-transport chain. Cytochrome C also plays a role in apoptosis. Suppression of the anti-apoptotic members or activation of the pro-apoptotic members of the Bcl-2 family leads to altered mitochondrial outer membrane permeability resulting in release of cytochrome C into the cytosol. Binding of cytochrome C to Apaf-1 triggers the activation of caspase-9, which then accelerates apoptosis by activating other caspases.

Platform Microplate (12 x 8 well strips)

Properties

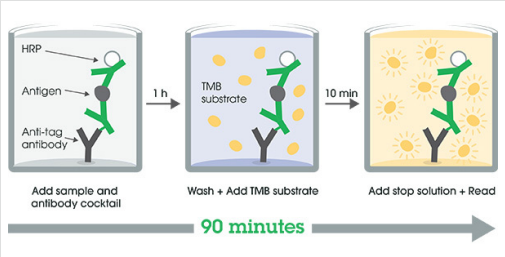
Storage instructions Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Human Cytochrome C Capture Antibody	1 x 600µl
10X Human Cytochrome C Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent CPI - HAMA Blocker (ab193969)	1 x 6ml
Denaturant	1 x 500µl
Human Cytochrome C Lyophilized Recombinant Protein	1 x 2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 12ml

Components	1 x 96 tests
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

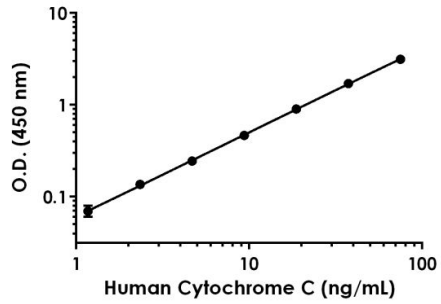
Function	Electron carrier protein. The oxidized form of the cytochrome c heme group can accept an electron from the heme group of the cytochrome c1 subunit of cytochrome reductase. Cytochrome c then transfers this electron to the cytochrome oxidase complex, the final protein carrier in the mitochondrial electron-transport chain. Plays a role in apoptosis. Suppression of the anti-apoptotic members or activation of the pro-apoptotic members of the Bcl-2 family leads to altered mitochondrial membrane permeability resulting in release of cytochrome c into the cytosol. Binding of cytochrome c to Apaf-1 triggers the activation of caspase-9, which then accelerates apoptosis by activating other caspases.
Involvement in disease	Defects in CYCS are the cause of thrombocytopenia type 4 (THC4) [MIM:612004]; also known as autosomal dominant thrombocytopenia type 4. Thrombocytopenia is the presence of relatively few platelets in blood. THC4 is a non-syndromic form of thrombocytopenia. Clinical manifestations of thrombocytopenia are absent or mild. THC4 may be caused by dysregulated platelet formation.
Sequence similarities	Belongs to the cytochrome c family.
Post-translational modifications	Binds 1 heme group per subunit.
Cellular localization	Mitochondrion matrix.

Images



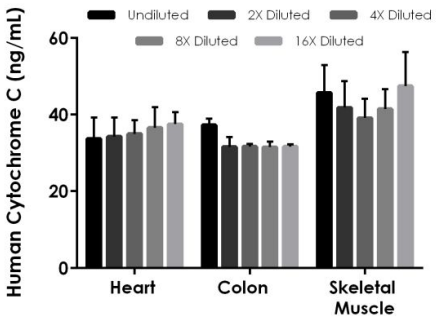
Other - Human Cytochrome C ELISA Kit (ab221832)

SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



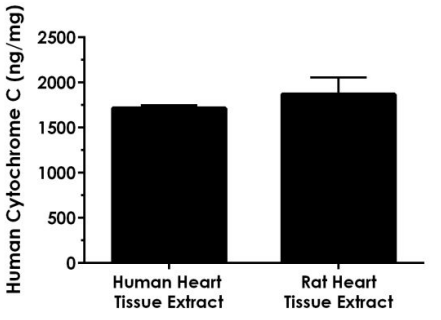
Human Cytochrome C standard curve

Example of human Cytochrome C standard curve in 1X Cell Extraction Buffer PTR + Enhancer.



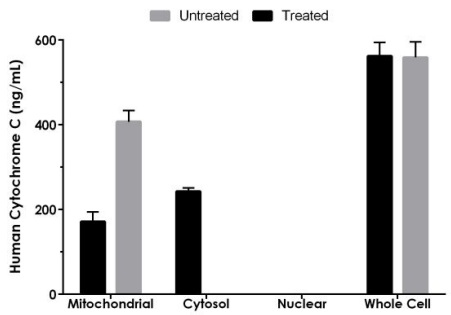
Interpolated concentrations of native Cytochrome C in human heart tissue extract

Interpolated concentrations of native Cytochrome C in human heart tissue extract based on a 20 µg/mL extract load, colon tissue extract based on a 50 µg/mL extract load, and skeletal muscle tissue extract based on a 25 µg/mL extract load. The concentrations of Cytochrome C were measured in duplicate and interpolated from the Cytochrome C standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Cytochrome C concentration was determined to be 35.41 ng/mL in heart tissue extract, 32.74 ng/mL in colon tissue extract, and 43.12 ng/mL in skeletal muscle tissue extract.



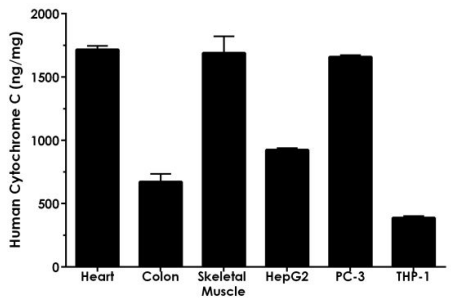
Reactivity in human and rat heart tissue extracts

Other species reactivity was determined by measuring a 20 □g/mL extract load of human and rat heart tissue extract samples, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in the human heart tissue extract. Cross-reactivity was determined to be 100% in rat heart extract. Due to 100% amino acids sequence identity of rat and mouse Cytochrome C, the same cross-reactivity can be assumed for mouse Cytochrome C.



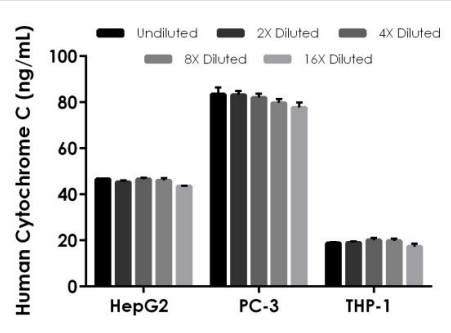
Cytochrome C distribution in subcellular fractions

Comparison Cytochrome C distribution in subcellular fractions derived from 3.7x103 HeLa cells and whole cells cultured in the presence (treated) or absence (untreated) of 1 µM staurosporine for 4 hours. Cells were collected directly after treatment and subcellular fractions were prepared using a cell fractionation kit (ab109719). Fractions were processed as described in section 11.10. and assayed. The concentrations of Cytochrome C were measured in three different dilutions of the fraction samples in duplicates and interpolated from the Cytochrome C standard curve. The interpolated values are plotted (mean +/- SD, n=3). The mean Cytochrome C concentration was determined be 171.4 ng/mL in the treated cytosol fraction, 242.8 in the treated mitochondrial fraction, 562.0 in the treated whole cell sample, 407.2 ng/mL in the untreated mitochondrial fraction, and 558.9 ng/mL in the untreated whole cell sample. Cytochrome C was not detectable in the untreated cytosol fraction and in both nuclear fractions.



Interpolated concentrations of native Cytochrome C in human extract samples

Interpolated concentrations of native Cytochrome C in human extract samples. The concentrations of Cytochrome C were measured in three different dilutions in duplicate and interpolated from the Cytochrome C standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted in ng of Cytochrome C per mg of extract (mean +/- SD, n=3). Cytochrome C concentration was determined to be 1716 ng/mg heart tissue extract, 670.1 ng/mg in colon tissue extract, 1689 ng/mg in skeletal muscle tissue extract, 924.2 ng/mg in HepG2 cell extract, 1658 ng/mg in PC-3 cell extract, and 386.2 ng/mg in THP-1 cell extract samples.



Interpolated concentrations of native Cytochrome C in HepG2 cell extract, PC-3 cell extract, and THP-1 cell extract

Interpolated concentrations of native Cytochrome C in HepG2 cell extract, PC-3 cell extract, and THP-1 cell extract samples based on a 50 µg/mL extract load. The concentrations of Cytochrome C were measured in duplicate and interpolated from the Cytochrome C standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Cytochrome C concentration was determined to be 45.64 ng/mL in HepG2 cell extract, 81.23 ng/mL in PC-3 cell extract, and 19.01 ng/mL in THP-1 cell extract.

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