abcam

Product datasheet

Human Cytochrome C ELISA Kit ab221832

SimpleStep ELISA

4 References 8 Images

Overview

Product name Human Cytochrome C ELISA Kit

Detection methodColorimetric

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 Human Cytochrome C ELISA Kit (ab221832) is a single-wash 90 min sandwich ELISA designed

for the quantitative measurement of Cytochrome C protein in cell culture extracts and tissue extracts. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human Cytochrome

C with 1100 pg/ml sensitivity.

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SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (<u>ab203359</u>) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

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.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.

It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

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

Components	1 x 96 tests
Human Cytochrome C Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 12ml
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 proapoptotic 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.

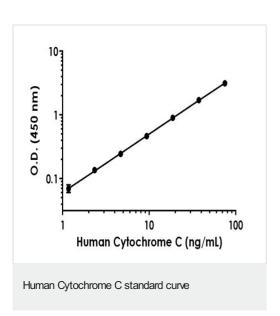
Post-translational modifications

Binds 1 heme group per subunit.

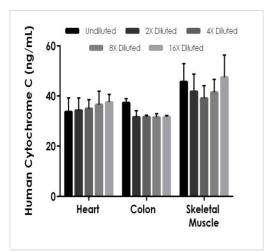
Cellular localization

Mitochondrion matrix.

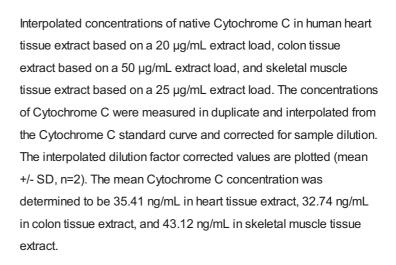
Images

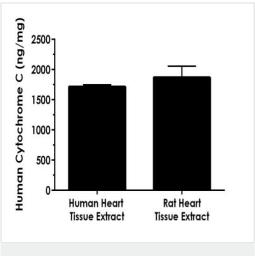


Example of human Cytochrome C standard curve in 1X Cell Extraction Buffer PTR + Enhancer.



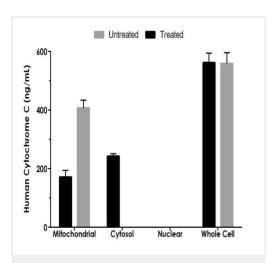
Interpolated concentrations of native Cytochrome C in human heart tissue extract





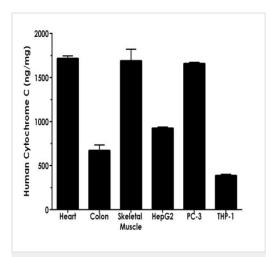
Reactivity in human and rat heart tissue extracts

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.



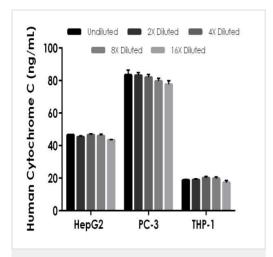
Cytochrome C distribution in subcellular fractions

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.



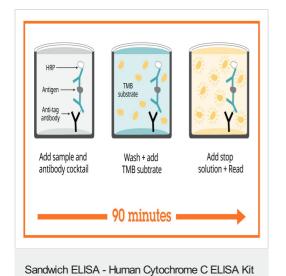
Interpolated concentrations of native Cytochrome C in human extract samples

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.



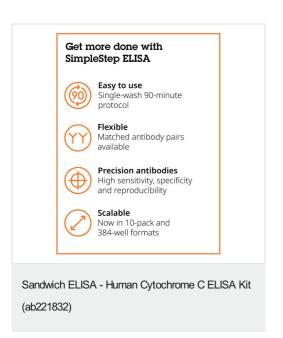
Interpolated concentrations of native Cytochrome C in HepG2 cell extract, PC-3 cell extract, and THP-1 cell extract

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.



(ab221832)

SimpleStep ELISA technology allows the formation of the antibodyantigen 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.



To learn more about the advantages of SimpleStep ELISA® kits see <u>here</u>.

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