Human Cytochrome C ELISA Kit

Instructions for use

Catalogue numbers: 1x96 tests: 650.070.096
2x96 tests: 650.070.192

For research use only

Fast Track Your Research…….

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Human Cytochrome C ELISA KIT

1. Intended use

The Cytochrome C (Cyt C) ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human cytochrome C in cell culture lysates, human whole blood or serum.

This kit has been configured for research use only.

2. Introduction

2.1. Summary

Apoptotic cell death is a fundamental feature of virtually all cells (5). It is an indispensable process during normal development, tissue homeostasis, development of nervous system and regulation of the immune system. Insufficient or excessive cell death can contribute to human disease, including cancer or degenerative disorders (14). The highly coordinated and stereotyped manner of induced cell death suggests that cells activate a common death program, towards which diverse signal – transducing pathways converge (2,17,18).

The mitochondria turned out to participate in the central control or executioner phase of the death cascade (1). Cytochrome C was identified as a component required for crutial steps in apoptosis, caspase-3 activation and DNA fragmentation (8). Cytochrome C was shown to redistribute from mitochondria to cytosol during apoptosis in intact cells (6a, b).

Mitochondrial cytochrome C is a water – soluble protein of 15 kDa with a net positive charge, residing loosely attached in mitochondrial intermenbrane space. Cytochrome C functions in respiratory chain by interaction with redox partners. It is highly conserved during evolution. Like most mitochondrial proteins cytochrome C is encoded by a nuclear gene and synthesized as cytoplasmic precursor molecule, apocytochrome c, which becomes selectively imported into the mitochondrial intermembran space. The molecular mechanisms responsible for the translocation of cytochrome C from mitochondria to cytosol during apoptosis are unknown.
A reduction in mitochondrial transmembrane potential has been reported to accompany early apoptosis (7). The release of cytochrome C into cytosol leads to an activation of an apopototic program via activation of a caspase dependent pathway (12,15,13,4). Cytochrome C achieves this goal by interaction with other cytosolic factors forming a complex (apoptosome) composed of cytochrome C, Apaf-1,dATP and Apaf-3/caspase 9 (10,11,3). Bcl-2 on the other hand was shown to be able to prevent apoptosis by blocking the release of cytochrome c from mitochondria (18).

Measurment of cytochrome C release from mitochondria is tool to detect the first early steps for initiating apoptosis in cells. Cytochrome C release in cytosol occurs prior to the activation of caspases and DNA fragmentation which is considered the hallmark of apoptosis.
Detection of cytochrome c release from the mitochondria to the cytoplasm can be achieved by selective lysis of cell membrane.
Very recently it has been shown that the mitochondria dwelling molecule can be detected in medium already 1 h after apoptosis. Moreover, elevated cytochrome C levels were observed in serum from patients with hematological malignancies. In course of cancer chemotherapy, the serum-cytochrome C level grew rapidly and it decreased gradually as the patient was cleared from malignant cells. Thus, serum-cytochrome C monitoring might serve as a clinical marker indicating the onset of apoptisis and cell turn-over in vivo (9)

2.2. Principle of the method

A capture Antibody highly specific for Cyt C has been coated to the wells of the microtiter strip plate provided during manufacture. Binding of Cyt C in samples and known standards to the capture antibodies and subsequent binding of the biotinylated anti-Cyt C secondary antibody to the analyte is completed during the same incubation period. Any excess unbound analyte and secondary antibody is then removed. The HRP conjugate solution is then added to every well including the zero wells, following incubation excess conjugate is removed by careful washing. A chromogen substrate is added to the wells resulting in the progressive development of a blue coloured complex with the conjugate. The colour development is then stopped by the addition of acid turning the resultant final product yellow. The intensity of the produced coloured complex is directly proportional to the concentration of Cyt C present in the samples and standards. The absorbance of the colour complex is then measured and the generated OD values for each standard are plotted against expected concentration forming a standard curve. This standard curve can then be used to accurately determine the concentration of Cyt C in any sample tested.

3. Reagents provided and reconstitution

REAGENTS (store at 2-8°C)	Quantity 1x96 well kit Cat no. 650.070.096	Quantity 2x96 well kit Cat no. 650.070.192	RECONSTITUTION
96 well microtiter strip plate	1	2	Ready-to-use
Plate covers	2	4	
Cytochrome C Standard: 10 ng /ml	2 vials	4 vials	Reconstitute with the volume of distilled water indicated on the vial
Biotin-Conjugate anti cytochrome C	1 vial	2 vials	(0.1ml) Dilute 100 times in Assay Buffer
Streptavidin-HRP	1 vial	2 vials	(150 µl) Dilute 200 times in Assay Buffer
Assay Buffer Concentrate	1 vial	2 vials	(5 ml) 20X concentrate. Dilute in distilled water
Wash Buffer Concentrate	1 vial	2 vials	(50 ml) 20X concentrate. Dilute in distilled water
Lysis buffer	1 vial	2 vials	(15 ml), 10X. Dilute in distilled water
Substrate Solution	1 vial	2 vials	(15 ml) Ready-to-use
Stop Solution (1 M Phosphoric acid)	1 vial	2 vials	(12 ml) Ready-to-use
Green Dye	1 vial	2 vials	(0.4 ml) Make a 1/100 dilution in the appropriate diluent
Red Dye	1 vial	2 vials	(0.4 ml) Make a 1/250 dilution in the appropriate diluent

4. Materials required but not provided

- Microtiter plate reader fitted with appropriate filters (450nm required with optional 630nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000µl adjustable single channel micropipettes with disposable tips
- 50-300µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile

5. Specimen collection, processing & storage

Cell culture supernatants, serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Cell culture supernatants: Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.

Storage: If not analyzed shortly after collection, samples should be aliquoted (250-500µl) to avoid repeated freeze-thaw cycles and stored frozen at –70°C. Avoid multiple freeze-thaw cycles of frozen specimens.

Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before use. When possible avoid use of badly haemolysed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

6. Safety & precautions for use

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures , e.g.CDC/NIH Health manual : " Biosafety in Microbiological and Biomedical Laboratories" 1984
- The human serum included in this kit have been tested and found non reactive for HbsAg, anti HIV1 & 2 and anti VHC. Nevertheless, no known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore handling of reagents, serum or plasma specimens should be in accordance with local safety procedures
- Laboratory gloves should be worn at all times
- Avoid any skin contact with H_2SO_4 and TMB. In case of contact, wash thoroughly with water
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used
- Do not pipette by mouth
- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels
- All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration
- Cover or cap all reagents when not in use
- Do not mix or interchange reagents between different lots
- Do not use reagents beyond the expiration date of the kit
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts
- Use a clean plastic container to prepare the washing solution
- Thoroughly mix the reagents and samples before use by agitation or swirling
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbance’s within 1 hour after completion of the assay
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells
- Follow incubation times described in the assay procedure
- Dispense the TMB solution within 15 min of the washing of the microtitre plate

7. Assay Preparation

Bring all reagents to room temperature before use

7.1. Assay Design

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard and zero should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the aluminium pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8^{o}C storage.

Example plate layout (example shown for a 6 point standard curve)

	Standards		Sample Wells									
	1	2	3	4	5	6	7	8	9	10	11	12
A	5	5										
B	2.5	2.5										
C	1.25	1.25										
D	0.63	0.63										
E	0.32	0.32										
F	0.16	0.16										
G	Blank	Blank										
H												

All remaining empty wells can be used to test samples in duplicate

7.2. Preparation of Wash Buffer

If crystals have formed in the Wash Buffer Concentrate, warm it gently until they have completely dissolved.

Pour entire contents (50 ml) of the **Wash Buffer Concentrate** into a clean 1,000 ml graduated cylinder. Bring final volume to 1,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. The pH of the final solution should adjust to 7.4.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that the Wash Buffer is stable for 30 days.

7.3. Preparation of Assay Buffer

Mix the contents of the bottle well. Add contents of **Assay Buffer Concentrate** (5.0 ml) to 95 ml distilled or deionized water and mix gently to avoid foaming. Store at 2° to 8°C. Please note that the Assay Buffer is stable for 30 days.

7.4. Preparation of Standard

Standard vials must be reconstituted with the volume of distilled water shown on the vial immediately prior to use. This reconstitution gives a stock solution of 10ng/ml of CytC. **Mix the reconstituted standard gently by inversion only**. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 5 to 0.16 ng/ml. A fresh standard curve should be produced for each new assay.

- Add 100ml of Assay buffer to all standard and blank wells
- Immediately after reconstitution add 100µl of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 5ng/ml. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells
- Transfer 100µl from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells
- Continue this 1:1 dilution using 100µl from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 5ng/ml to 0.16ng/ml
- Discard 100µl from the final wells of the standard curve (F1 and F2)

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred directly into the relevant wells.

7.5. Preparation of Samples

Cell Lysis procedure (**cell culture sample**)

a. Spin down cells for 15 minutes at 200 x g.
b. Wash cell pellet once in cold PBS
c. Re-suspend cells in Lysis buffer to a concentration of 1.5 x 10^6 cells/ml.
d. Incubate for 1 hour at room temperature with gentle shaking.
e. Centrifuge cells at 1000 x g for 15 minutes.
f. Dilute the supernatant in assay buffer for the assay at least 50 –fold (5µl supernatant + 245 µl Assay Buffer) for assay. Aliquot and store supernatant not used immediately at -70°C

Cell Lysis procedure (**whole blood samples**)

a. Spin down 1 ml of whole blood for 15 minutes at 200 x g.
b. Remove plasma (supernatant) carefully.
c. Resuspend cell pellet in 3 ml Lysis buffer.
d. Incubate for 1 hour at room temperature with gently shaking.
e. Spin down for 15 minutes at 1000 x g.
f. Dilute the supernatant at least 10 –fold in Assay Buffer and assay immediately. Aliquot supernatant not needed and store at -70°C.

Serum samples dilute serum samples before assaying 1:2 in assay buffer (e.g 150 µl serum sample 150 µl assay buffer)

Mix all reagents thoroughly without foaming before use.

7.6. Preparation of Biotin Conjugate

Make a 1:100 dilution with **Assay Buffer** (reagent B) in a clean plastic tube as needed according to the following table :

Number of Strips	Biotin-Conjugate (µl)	Assay Buffer (ml)
1 - 6	30	2.97
1 - 12	60	5.94

7.7. Preparation of Streptavidin-HRP

Make a 1:200 dilution with Assay Buffer of the concentrated **Streptavidin-HRP** solution as needed according to the following table:

Number of Strips	Streptavidin-Conjugate (µl)	Assay Buffer (ml)
1 - 6	30	5.97
1 - 12	60	11.94

7.8. Preparation of Lysis Buffer

Add contents of Lysis buffer concentrate (15ml) to 135 ml distilled or desionized water and mix gently. Store at room temperature.

7.9. Addition of Color Dyes

In order to help our customers to avoid any mistakes in pipetting, color dyes help to monitor the addition of even very small volumes of a solution to the reaction well by giving distinctive colours to each step of the ELISA procedure.

This procedure is optional, does not in any way interfere with the test results, and is designed to help the customer with the performance of the test, but can also be omitted, just following the instruction booklet.
Alternatively, the dye solutions from the stocks provided (***Green-Dye, Red Dye***) can be added to the reagents according to the following guidelines:

A. Biotin-Conjugate:

Before dilution of the concentrated conjugate, add the ***Green-Dye*** at a dilution of 1:100 (see table below) to the Assay Buffer (1x) used for the final conjugate dilution. Proceed after addition of ***Green-Dye*** according to the instruction booklet, preparation of Biotin-conjugate.

3 ml Assay Buffer	30 µl ***Green-Dye***
6 ml Assay Buffer	60 µl ***Green-Dye***
12 ml Assay Buffer	120 µl ***Green-Dye***

B. Streptavidin-HRP:

Before dilution of the concentrated Streptavidin-HRP, add the ***Red-Dye*** at a dilution of 1:250 (see table below) to the Assay Buffer (1x) used for the final Streptavidin-HRP dilution. Proceed after addition of ***Red-Dye*** according to the instruction booklet, preparation of Streptavidin-HRP.

6 ml Assay Buffer	24 µl ***Red-Dye***
12 ml Assay Buffer	48 µl ***Red-Dye***

8. Method

We strongly recommend that every vial is mixed thoroughly without foaming prior to use except the standard vial which must be mixed gently by inversion only.

Prepare all reagents as shown in section 7.

Assay Step		Details
1.	Wash	Remove the pre-coated plate from the sealed pouch, removed any un-needed strips and wash the plate as follows: a) Dispense 0.3 ml of **1x washing solution** into each well b) Aspirate the contents of each well c) Repeat step a and b
2.	Addition	**Prepare Standard curve** as shown in section 7.4
3.	Addition	Add 100µl of each **standard, sample and zero** in duplicate to appropriate number of wells
4.	Addition	Add 50µl of diluted **biotinylated Conjugate** to all wells
5.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **2 hours**
6.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.3 ml of **1x washing solution** into each well c) Aspirate the contents of each well d) Repeat step b and c another two times
7.	Addition	Add 100µl of **Streptavidin-HRP** solution into all wells
8.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour**
9.	Wash	Repeat wash step 6.
10.	Addition	Add 100µl of ready-to-use **TMB Substrate Solution** into all wells
11.	Incubation	Incubate in the dark for **10 minutes*** at room temperature on a rotar set at 100rpm if available. Avoid direct exposure to light by wrapping the plate in aluminium foil
12.	Addition	Add 100µl of **H_2SO_4:Stop Reagent** into all wells
Read the absorbance value of each well (immediately after step 12.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 630 nm as the reference wave length (610 nm to 650 nm is acceptable).		

***Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range*

9. Data Analysis

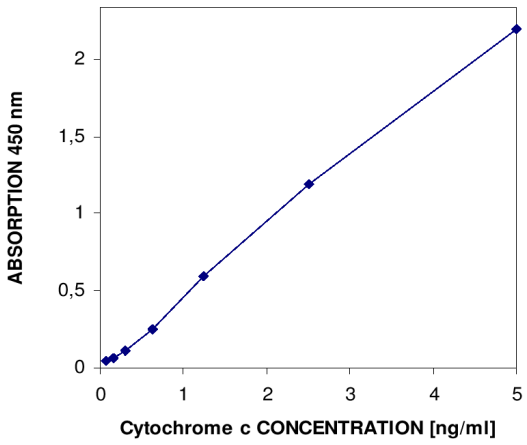
Calculate the average absorbance values for each set of duplicate standards and samples**.** Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding CytC standard concentration on the horizontal axis.

The amount of CytC in each sample is determined by extrapolating OD values against CytC standard concentrations using the standard curve**.**

Example Cytochrome C Standard Curve

Standard	Cyto C Conc (ng/ml)	OD (450nm) mean	CV (%)
1	5	2.196	5.8
2	2.5	1.187	2.2
3	1.25	0.594	4.6
4	0.63	0.254	5.5
5	0.31	0.112	12.6
6	0.16	0.066	5.3
Zero	0	0.029	-



Note; curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

For samples which have been diluted according to the protocol, the calculated concentration should be multiplied by the dilution factor.

10. Assay limitations

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results.

Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh Washing Buffer, fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

11. Performance Characteristics

11.1. Sensitivity

The limit of detection of cytochrome C defined as the analyte concentration resulting in an absorption significantly higher than that of the dilution medium (mean plus two standard deviations) was determined to be less than 0.05 ng/ml (mean of 6 independent assays).

11.2. Specificity

The interference of circulating factors of the immune systems was evaluated by spiking several of these proteins at physiologically relevant concentrations into a Cytochrome c positive serum. There was no detectable cross reactivity.

11.3. Precision

Intra-assay

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with 6 replicates of 8 samples containing different concentrations of cytochrome c. Two standard curves were run on each plate. Data below show the mean Cytochrome C concentration and the coefficient of variation for each sample. The **overall intra-assay coefficient of variation has been calculated to be 6.0 %.**

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments by three technicians. Each assay was carried out with 6 replicates of 8 samples containing different concentrations of cytochrome c. Two standard curves were run on each plate. Data below show the mean Cytochrome C concentration and the coefficient of variation calculated on 18 determinations of each sample. **The overall inter-assay coefficient of variation has been calculated to be 3.96 %.**

11.4. Dilution Parallelism

Four cell lystates with different levels of cytochrome c were assayed at four serial two-fold dilutions with 4 replicates each. In the table below the percent recovery of expected values is listed. Recoveries ranged from 78 % to 119 % **with an overall mean recovery of 98 %.**

11.5. Spike Recovery

The spike recovery was evaluated by spiking three levels of cytochrome c into cells lysates. Recoveries were determined in three independent experiments with 6 replicates each. Recoveries ranged from 78 % to 88 % with an **overall mean recovery of 82 %.**

11.6. Stability

Storage Stability
Aliquots of a serum sample (spiked or unspiked) were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the cytochrome c level determined after 24 h. There was a significant loss of Cytochrome C immunoreactivity during storage at above conditions.

Freeze-thaw Stability
Aliquots of serum samples (unspiked or spiked) were stored frozen at -20°C and thawed up to 5 times, and cytochrome c levels determined. There was a significant loss of cytochrome c by freezing and thawing up to 5 cycles freezing and thawing

12. Bibliography

1) Bossy-Wetzel, E., Newmeyer, D.D. and Green, D.R. (1998). Mitochondrial cytochrome c release in apoptosis occurs upstream of DEVD-specific caspase activation and independently of mitochondrial transmembrane depolarization.
EMBO J., 17, 37-49.

2) Chinnaiyan, A. M. and Dixit, V.M. (1996). The cell-death machine.
Curr. Biol., 6, 555-562.

3) Green, D.R. and J.C. Reed (1998). Mitochondria and apoptosis.
Science 281:1309.

4) Hakem, R., Hakem A, Duncan GS, Henderson JT, Woo M, Soengas MS, Elia A, de la Pompa JL, Kagi D, Khoo W, Potter J, Yoshida R, Kaufman SA, Lowe SW, Penninger JM, Mak TW (1998). Differential requirement for caspase 9 in apoptotic pathways in vivo.
Cell 94(3):339-52.

5) Jacobson, M.D., Weil, M. and Raff, M.C. (1997). Programmed cell death in animal development

6a) Kluck, R.M., Bossy-Wetzel, E., Green, D.R. and Newmeyer, D.D. (1997a). The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. Science, 275, 1132-1136.

b) Kluck, R.M., Martin, S.J., Hoffman, B.M., Zhou,J.S., Green, D.R. and Newmeyer, D.D. (1997b) Cytochrome c activation of CPP32-like proteolysis plays a critical role in a Xenopus cell-free apoptosis system.
EMBO J.,16, 4639-4649.

7) Kroemer, G. (1997b). The proto-oncogene Bcl-2 and its role in regulating apoptosis.
Nature Med., 3, 614-620.

8) Liu, X., Kim, C.N., Yang, J. Jemmerson, R. and Wang, X. (1996). Induction of the apoptotic program in cell-free extracts: requirement for dATP and cytochrome c.
Cell,86, 147-157.

9) Los, M., Renz A, Berdel WE, Kreuter M and Schulze-Osthoff K. (2000). Cytochrome c is rapidly released from the cell upon apoptosis induction: a new marker for cell death in vivo.
IFES Congress, Poland 2000.

10) Reed, J.C., Jurgensmeier J.M., Matsuyama S., (1998). Bcl-2 family proteins and mitochondria.
Biochem. Biophys. Acta 1366(1-2):127-37.

11) Reed, J.C. (1997). Cytochrome c: can`t live with it - can`t live without it.
Cell 91(5):559-62.

12) Skulachev, V.P., (1998). Cytochrome c in the apoptotic and antioxidant cascades. FEBS Lett. 423(3):275-80.

13) Slee, E.A., Harte M.T., Kluck RM, Wolf B.B., Casiano C.A., Newmeyer D.D., Wang H.G., Reed J.C., Nicholson D.W., Alnemri E.S., Green D.R., Martin S.J. (1999). Ordering the cytochrome c-initiated caspase cascade: hierarchical activation of caspases-2,-3,-6,-7,-8, and –10 in a caspase-9-dependent manner.
J. Cell Biol. 144(2):281-92.

14) Thompson, C.B., (1995). Apoptosis in the pathogenesis and treatment of disease.
Science, 267, 1456-1462.

15) Tschopp, J., Thome M., Hofmann K., Meinl E., (1998). The fight of viruses against apoptosis.
Curr. Opin. Gen. Develop. 8(1):82-7.

16) Vaux, D.L. and Strasser , A. (1996). The molecular biology of apoptosis.
Proc. Natl Acad. Sci. USA,83, 2239-2244.

17) White, E., (1996). Life, death and the pursuit of apoptosis.
Genes Dev., 10, 1-15.

18) Yang, J., Liu, X., Bhalla, K., Kim, C.N., Ibrado, A.M., Cai, J., I Peng, T., Jones, D.P., Wang X., (1997). Prevention of Apoptosis by Bcl-2: Release of Cytochrome c from Mitochondrial Blocked.
Science, 275, 1129-32.

13. Assay Summary

Total procedure length : 3h10mn

Add 100µl sample and diluted standard and 50µl Biotinylated Conjugate

↓

Incubate 2 hours at room temperature

↓

Wash three times

↓

Add 100µl of Streptavidin-HRP

↓

Incubate 1 hour at room temperature

↓

Wash three times

↓

Add 100 µl of ready-to-use TMB
Protect from light. Let the color develop for 10 mn.

↓

Add 100 H_2SO_4

↓

Read Absorbance at 450 nm

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