Cytochrome c ELISA Kit

Catalog Number KHO1051 (96 tests)

Pub. No. MAN0014701 **Rev.** A.0 (31)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Cytochrome c ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of Cytochrome c in lysates of human, mouse, and rat cells.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHO1051 (96 tests)
Cytochrome c Standard; contains 0.1% sodium azide.	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide, red dye[1]	25 mL
Antibody Coated Plate; 96-well plate	1 plate
Cytochrome c Biotin Conjugate; contains 0.1% sodium azide, blue dye[1]	11 mL
Streptavidin-HRP (100X)	0.15 mL
HRP Diluent; contains 3.3 mM thymol, yellow dye[1]	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Adhesive Plate Covers	3

[1] To avoid pipetting mistakes, colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent are provided to monitor the addition of solution to each well. Dyes do not interfere with test results.

Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare Cell Extraction Buffer

Note: See the *ELISA Technical Guide* for detailed information on preparing Cell Extraction Buffer.

1. Prepare Cell Extraction Buffer.

   Cell Extraction Buffer consists of 10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton™ X-100, 10% glycerol, 0.1% SDS, and 0.5% deoxycholate.
2. Immediately before use, add PMSF (0.3 M stock in DMSO) to 1 mM and 50 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714) for each 1 mL of Cell Extraction Buffer.

Prepare cell lysate

1. Collect cells by centrifugation (non-adherent cells) or scraping from culture flasks (adherent cells), then wash cells twice with cold PBS.
2. Remove and discard the supernatant and collect the cell pellet. The pellet can be stored at −80°C and lysed at a later date if desired.
3. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes, on ice. Vortex at 10-minute intervals.

   Note: The volume of Cell Extraction Buffer used depends on the number of cells in the cell pellet, and expression levels of cytochrome c. Researchers must optimize the extraction procedures for their own applications.
4. Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
5. Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at −80°C (avoid multiple freeze-thaw cycles).

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

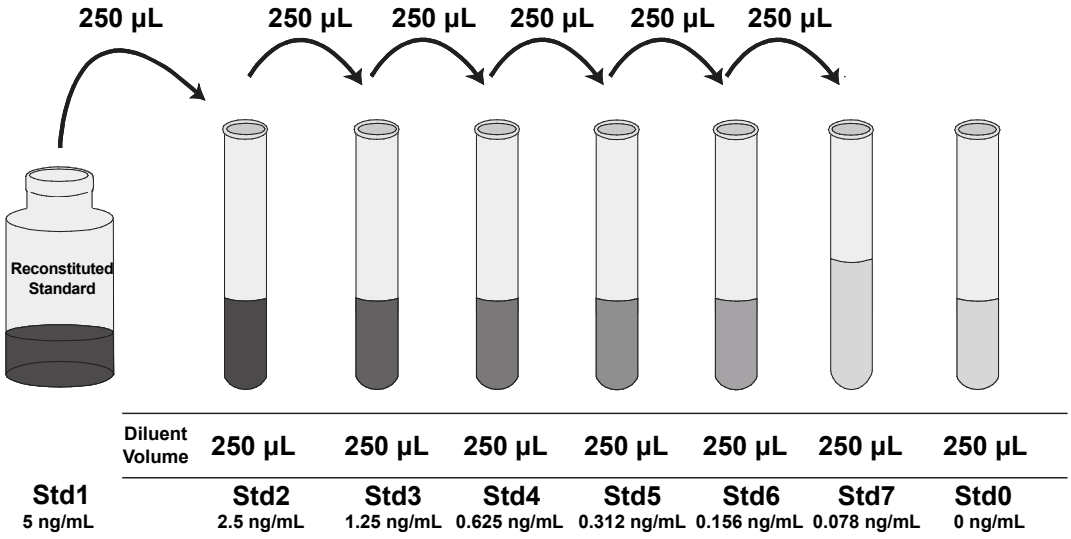
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

- Perform sample dilutions with Standard Diluent Buffer.
- Dilute samples prepared in Cell Extraction Buffer 1:10 or greater in Standard Diluent Buffer (e.g., 10 µL sample into 90 µL buffer). This dilution is necessary to reduce the matrix effect of the Cell Extraction Buffer. SDS concentration should be less than 0.01% before adding to the plate. While a 1:10 sample dilution has been found to be satisfactory, higher dilutions such as 1:50 or 1:100 may be optimal.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Cytochrome c Standard to 5 ng/mL with Standard Dilution Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 5 ng/mL cytochrome c. **Use the standard within 1 hour of reconstitution.**
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0 ng/mL cytochrome c.
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Discard any remaining reconstituted standard or freeze in aliquots at −80°C. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

To ensure accurate dilution:

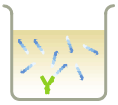
1. For each 8-well strip used in the assay, pipet 10 µL of Streptavidin-HRP (100X) solution, then dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen



a. Add 100 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Cytochrome c Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 5 ng/mL cytochrome c.

Standard Cytochrome c (ng/mL)	Optical Density (450 nm)
5	3.44
2.5	1.86
1.25	0.85
0.625	0.42
0.312	0.26
0.156	0.17
0.078	0.15
0	0.12

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	2.42	1.20	0.61
Standard Deviation	0.15	0.06	0.05
% Coefficient of Variation	6.20	5.25	8.77

Intra-assay precision

Samples of known cytochrome cconcentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	2.45	1.21	0.55
Standard Deviation	0.09	0.06	0.03
% Coefficient of Variation	3.71	4.61	5.45

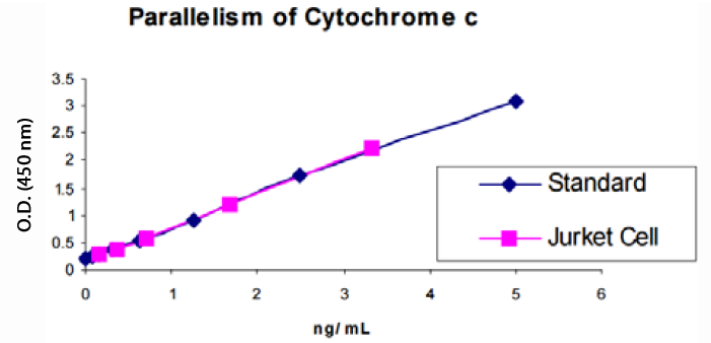
Linearity of dilution

Jurket cells were grown in cell culture medium containing 10% fetal bovine serum and lysed with Cell Extraction Buffer. The lysate was diluted in Standard Diluent Buffer over the range of the assay and measured for cytochrome c. Linear regression analysis of sample values versus the expected concentrations yielded a correlation coefficient of 0.99.

Dilution	Cell Lysate		
	Measured (ng/mL)	Expected	
		(ng/mL)	%
1/2	3.33	3.33	100
1/4	1.67	1.66	100
1/8	0.72	0.83	86
1/16	0.37	0.42	88

Parallelism

Natural cytochrome c from Jurket cell lysate was serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the cytochrome c standard curve. The standard accurately reflects cytochrome c content in samples.



Recovery

To evaluate recovery, Cytochrome c ELISA Kit Standard was spiked at 3 different concentrations into 10% cell extraction buffer. The average recovery was 110%.

Sensitivity

The analytical sensitivity of cytochrome c is <0.156 ng/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 24 times, and calculating the corresponding concentration.

Specificity

Various human, rat, and mouse cell lines were tested on the Cytochrome c ELISA Kit and it was found that the assay can detect human, rat, and mouse forms of the cytochrome c protein.

Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.