Human c-Myc (Total) ELISA Kit

Catalog Number KHO2041 (96 tests)

Pub. No. MAN0003782 **Rev.** 4.0 (30)

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 Invitrogen™ Human c-Myc (Total) ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the total level of human c-Myc in human cell lysates. The assay recognizes both natural and recombinant human c-Myc.

The c-Myc protein is a 57 kDa transcription factor that activates the transcription of target genes that include cyclins and translation initiation factors. Like other Myc transcription factors, c-Myc has a helix-loop-helix leucine zipper motif at the carboxyl-terminal domain. At the amino-terminal domain, there are two highly conserved regions required for the activation of target genes.

Contents and storage

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

Contents	Cat. No. KHO2041 (96 tests)
Hu c-Myc (Total) Standard; lyophilized. Refer to vial label for reconstitution volume	1 vial
Standard Diluent Buffer; contains 0.1% sodium azide and red dye[1]	25 mL
Hu c-Myc (Total) Antibody Coated Plate; 96-well plate	1 plate
Rabbit anti-Hu c-Myc Detection Antibody; contains 0.1% sodium azide and blue dye[1]	6 mL
Anti-Rabbit IgG HRP (100X); contains 3.3 mM thymol	0.125 mL
HRP Diluent; contains 3.3 mM thymol and yellow dye[1]	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

[1] To help monitor the addition of reagents to the reaction wells and avoid any pipetting errors, we provide colored Standard Diluent Buffer, Detection Antibody, and HRP Diluent. The colored dye does not interfere with the test results.

Required materials not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Cell Extraction Buffer (Cat. No. FNN0011, or see "Prepare Cell Extraction Buffer")

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 human c-Myc. FOR EXAMPLE, 10^8 HeLa cells can be extracted in 1 mL of Cell Extraction Buffer. 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. Sonicate the lysate 3 times (10–15 seconds each time). Incubate the lysate on ice between sonication to ensure it stays cold
6. Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at −80°C (avoid multiple freeze-thaw cycles).

Pre-dilute samples

Because conditions may vary, we recommend that each investigator 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., 5 µL sample into 45 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 1:25 or 1:50 may be optimal.

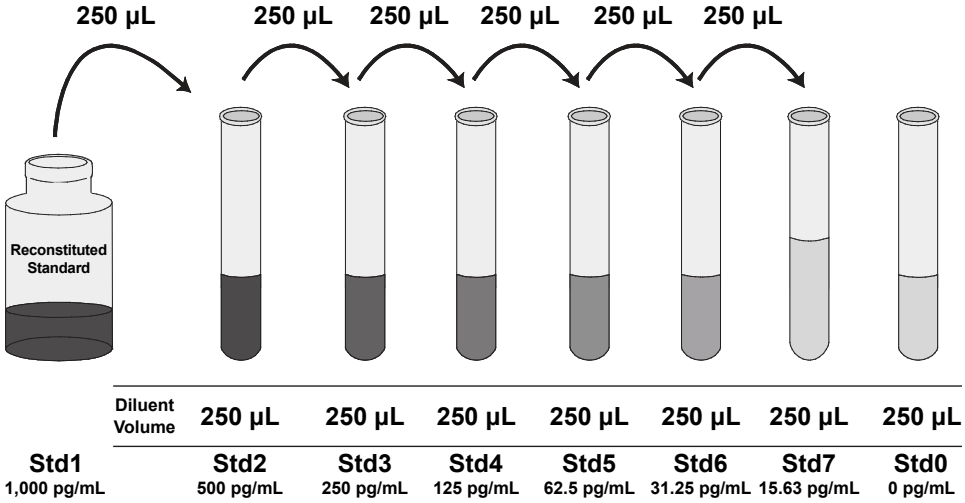
  For 10^8 HeLa cells use 1−10 µL of the clarified cell lysate diluted to 50 µL/well in Standard Diluent Buffer for each well.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: The Hu c-Myc (Total) Standard is prepared using purified c-Myc recombinant protein.

1. Reconstitute Hu c-Myc (Total) Standard to 1,000 pg/mL with Standard Diluent 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 1,000 pg/mL human c-Myc. **Use the standard within 1 hour of reconstitution.**
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 500, 250, 125, 62.5, 31.25, 15.63, and 0 pg/mL human c-Myc.
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare 1X Anti-Rabbit IgG HRP solution within 15 minutes of usage.

The Anti-Rabbit IgG HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG 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 and add detector

a. Add 50 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. Add 50 µL of Hu c-Myc (Total) Detection Antibody solution into each well except the chromogen blanks.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate 3 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add IgG HRP

a. Add 100 µL 1X Anti-Rabbit IgG HRP solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 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.

4 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 4 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 0 to 1,000 pg/mL human c-Myc.

Standard Human c-Myc (Total) (pg/mL)	Optical Density (450 nm)
1,000	2.46
500	1.48
250	0.79
125	0.41
62.5	0.21
31.25	0.11
15.63	0.06
0	0.02

Inter-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	2.0	0.6	0.2
Standard Deviation	0.1	0.02	0.01
% Coefficient of Variation	3.3	3.5	4.3

Intra-assay precision

Samples of known human c-Myc concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	29.8	120.0	473.9
Standard Deviation	1.9	4.5	20.2
% Coefficient of Variation	6.7	3.7	4.3

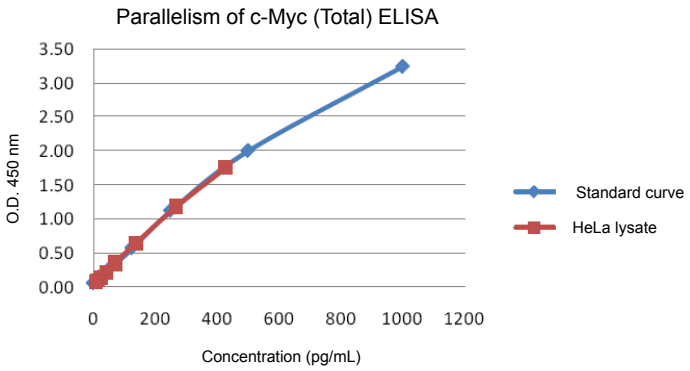
Linearity of dilution

HeLa cells were grown in DMEM with 10% fetal bovine serum, lysed with Cell Extraction Buffer, and sonicated. The lysate was diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Measured (pg/mL)	Expected	
		(pg/mL)	%
1/10	372.3	372.3	100
1/20	205.7	186.14	111
1/40	117.2	93.07	126
1/80	56.0	46.54	120
1/160	25.8	23.27	111
1/320	8.8	11.6	76

Parallelism

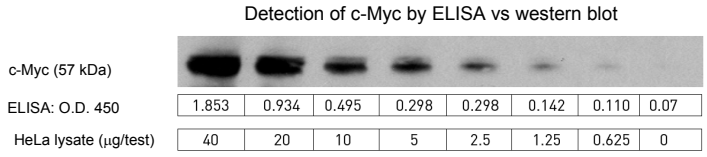
Natural human c-Myc from HeLa cell lysate was serially diluted in Standard Diluent Buffer. The expected concentration of each dilution was plotted against the optimal density and compared to the c-Myc standard curve.



Sensitivity

The analytical sensitivity of this assay is <5 pg/mL of human c-Myc. This was determined by adding two standard deviations to the mean O.D. obtained from 30 assays of the zero standard.

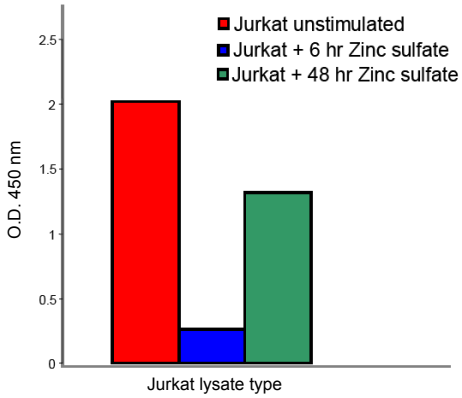
The sensitivity of this ELISA is similar to that of western blot when tested against known quantities of human c-Myc.



ELISA: O.D. 450	1.853	0.934	0.495	0.298	0.298	0.142	0.110	0.07
HeLa lysate (µg/test)	40	20	10	5	2.5	1.25	0.625	0

Specificity

The Human c-Myc (Total) ELISA Kit is specific for the measurement of the total amount of human c-Myc. To determine the specificity, same concentrations of cell extracts from different Jurkat preparations were analyzed.



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

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.