Performance characteristics

Intra-assay precision

Samples of known 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
SD	1.9	4.5	20.2
%CV	6.7	3.7	4.3

SD = Standard Deviation; CV = Coefficient of Variation

Inter-assay precision

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

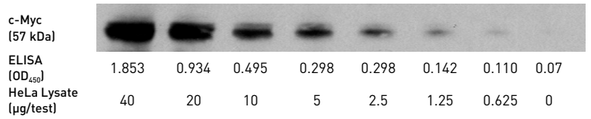
Parameters	Sample 1	Sample 2	Sample 3
Mean (pg /mL)	2.0	0.6	0.2
SD	0.1	0.02	0.01
%CV	3.3	3.5	4.3

SD = Standard Deviation; CV = Coefficient of Variation

Sensitivity

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

The sensitivity of this ELISA was compared to western blotting using known quantities of c-Myc. The date presented below show that the sensitivity of the ELISA is similar to that of western blotting.



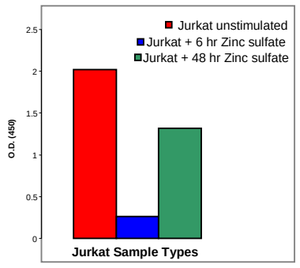
Linearity of dilution

HeLa cells were grown in DMEM (Cat. no. P104-500) containing 10% fetal bovine serum at 37°C, lysed with Cell Extraction Buffer, and sonicated. This lysate was diluted in Standard Diluent Buffer over the range of the assay and measured for c-Myc (total). Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

	Cell Lysate		
Dilution	Measured (pg/mL)	Expected (pg /mL)	% Expected
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

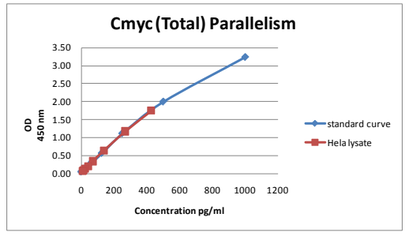
Specificity

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



Parallelism

Natural 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. Parallelism results indicate that the standard accurately reflects c-Myc content in samples.



Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

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

18 February 2013

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Human c-Myc (Total) ELISA Kit

Catalog. no. KHO2041 **Quantity:** 96 tests

Publication Part No. PRKHO2041 **Publication No:** MAN0003782 **Rev.** 3.00

Description

The Human c-Myc (Total) ELISA Kit is designed to detect and quantify the level of c-Myc protein independent of its phosphorylation state. This assay is intended for the detection of c-Myc in human cell lysates. Reactivity of this ELISA kit with other species is not assured.

The c-Myc protein is 57 kDa transcription factor. c-Myc activates the transcription of many target genes, including cell cycle cyclin D2 and CDK2, and translation initiation factors eIF2 and eIF4. Like other Myc transcription factors, n-Myc and L-Myc, 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. c-Myc's transcription activity involves complexes of several proteins. The protein MAX binds to the helix-loop-helix leucine zipper region of c-Myc. This dimerization with MAX triggers the binding of c-Myc-MAX to the DNA E-box sequence. Subsequently, c-Myc recruits histone acetyltransferase and other members of the chromatin remodeling complex. The amino-terminal domain plays an important role in the recruitment of the chromatin remodeling complex; one of the highly conserved regions in this domain binds with transformation/transcription domain-associated protein, a component of the remodeling complex. Another binding partner for the MAX protein is MAD. MAX-MAD heterodimer binds to the DNA sequences of c-Myc's target genes and represses the expression of these genes through the activity of histone deacetylases. Interactions between c-Myc, MAX, and MAD are critical in the control of cellular functions. When these interactions become unbalanced, such as the increased expression of c-Myc, tumors can develop. Elevated c-Myc expression is found in many cancer types.

Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

Components	Quantity
Hu c-Myc (Total) Standard. Lyophilized. Refer to vial label for quantity and reconstitution volume.	1 vial
Standard Diluent Buffer. Contains 0.1% sodium azide; red dye*.	25 mL
Hu c-Myc (Total) Antibody Coated Wells. 12 × 8 Well Strips.	1 plate
Rabbit anti-Hu c-Myc Detection Antibody. Contains 0.1% sodium azide; blue dye*.	6 mL
Anti-Rabbit IgG HRP (100X). Contains 3.3 mM thymol.	0.125 mL
HRP Diluent. Contains 3.3 mM thymol; yellow dye*	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

*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.

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support. This kit contains materials with small quantities of sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Materials required but not provided

- Cell Extraction Buffer (Cat. no. FNN0011)
- Distilled or deionized water
- Microtiter plate reader (at or near 450 nm) with software
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

Before starting

Review the **Procedural guidelines** and **Plate washing directions** in the ELISA Technical Guide available at www.lifetechnologies.com/manuals for details prior to starting the procedure.

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

For research use only. Not for use in diagnostic procedures.

Dilute wash buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 1 volume of the Wash Buffer Concentrate (25X) with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.
3. Store the concentrate and the Working Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare secondary antibody

Note: Prepare the secondary antibody within 15 minutes of usage.

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

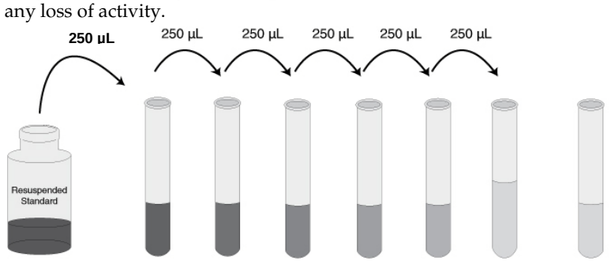
1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with a clean absorbent paper to remove any excess solution, and dispense the solution to a tube containing 1 mL of HRP Diluent.
2. Return the unused Anti-Rabbit IgG HRP (100X) to the refrigerator.

Dilute the standards

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

1. Reconstitute Hu c-myc (Total) Standard 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 1000 pg/mL c-Myc. Use the standard within 1 hour of reconstitution.
2. Add 0.25 mL Standard Diluent Buffer to each of 6 tubes labeled 500, 250, 125, 62.5, 31.25, and 15.63 pg/mL of c-Myc.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.

Aliquot and store any remaining reconstituted standard at –80ºC for further use. Standard can be frozen and thawed one time only without any loss of activity.



1000 pg/mL	500 pg/mL	250 pg/mL	125 pg/mL	62.5 pg/mL	31.25 pg/mL	15.63 pg/mL	0 pg/mL

Prepare cell lysate

The following extraction procedure is suitable for use with several cell lines using the Cell Extraction Buffer.

1. To the Cell Extraction Buffer (Cat. no. FNN0011), add the following protease inhibitors just prior to use:
   - 1 mM PMSF (stock is 0.3 M in DMSO)
   - Protease inhibitor cocktail (e.g. Sigma Cat. no. P-2714, reconstitute according to manufacturer's guideline). Add 500 µL per 5 mL Cell Extraction Buffer.

   Note: The stability of Cell Extraction Buffer with protease inhibitors is 24 hours at 4°C. PMSF is very unstable and must be added prior to use, even if added previously.
2. Collect cells in phosphate buffered saline by centrifugation (non-adherent) or scraping from culture flasks (adherent).
3. Wash cells twice with cold PBS. Discard the supernatant and collect the cell pellet. (At this point the cell pellet can be frozen at -80°C.)
4. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes on ice with vortexing at 10 minute intervals. The volume of Cell Extraction Buffer depends on the cell number in cell pellet and expression of c-Myc.

   For example, 10^8 HeLa cells grown in DMEM plus 10% FBS can be extracted in 1 mL of Cell Extraction Buffer. Under these conditions, use of 1-10 µL of the clarified cell lysate diluted to a volume of 50 µL/well in Standard Diluent Buffer is sufficient for the detection of c-Myc.
5. Sonicate the lysate 3 times for 10 to 15 seconds each time. Incubate the lysate on ice between sonications to ensure it stays cold.
6. Transfer lysates to microcentrifuge tubes and centrifuge at 13,793 × g for 10 minutes at 4°C.
7. Aliquot the clear lysates to clean microcentrifuge tubes for assay. Lysates can be stored at –80°C. Avoid multiple freeze-thaw cycles.

Dilute samples

Dilute samples 1:10 or greater in Standard Diluent Buffer (for example, dilute 10 µL sample into 90 µL buffer). This minimum 10-fold dilution step is necessary to avoid SDS interference with the assay. Optimize the dilution for each experimental system.

ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 4 hours.**

IMPORTANT! Perform a standard curve with each assay.

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.

Bind antigen/add detector antibody

1. Add 50 µL of standards and diluted samples (page 2) to the appropriate microtiter wells.
2. Add 50 µL of c-Myc Detection Antibody solution into each well.
3. Cover the plate with plate cover and incubate for 3 hours at room temperature.
4. Thoroughly aspirate the solution from the wells and wash wells 4 times with diluted Wash Buffer.

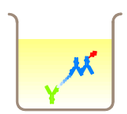
Add secondary antibody

5. Add 100 µL of diluted Anti-Rabbit IgG HRP (page 2) to each well.
6. Cover the plate with plate cover and incubate for 30 minutes at room temperature.
7. Thoroughly aspirate solution from wells and wash wells 4 times with diluted Wash Buffer.

Add chromogen

8. Add 100 µL of Stabilized Chromogen to each well.
9. Cover the plate with plate cover and incubate for 30 minutes at room temperature in the dark.

The substrate solution begins to turn blue. **Note:** TMB should not touch aluminum foil or other metals.



Add stop solution

10. Add 100 µL Stop Solution. Tap side of the plate gently 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: If samples produce signals greater than that of the highest standard, then dilute samples in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Standard curve (example)

The following data were obtained for the various standards over the range of 0 to 1000 pg/mL c-Myc (Total).

Standard Hu c-Myc (Total) pg/mL	Optical density (450 nm)
1000	2.46
500	1.48
250	0.79
125	0.41
62.5	0.21
31.25	0.11
15.625	0.06
0	0.02