

Store at
4°C
#45449C

PathScan® Total c-Myc Chemiluminescent Sandwich ELISA Kit

1 Kit (96 assays)



Support: +1-978-867-2388 (U.S.)
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Entrez-Gene ID #4609
UniProt ID #P01106

New 12/15

For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H

Description: The PathScan® Total c-Myc Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of c-Myc protein with a chemiluminescent readout. Chemiluminescent ELISAs often have a wider dynamic range and higher sensitivity than conventional chromogenic detection. This chemiluminescent ELISA, which is offered in low volume microplates, shows increased signal and sensitivity while using a smaller sample size. A c-Myc rabbit mAb has been coated on the microwells. After incubation with cell lysates, c-Myc proteins are captured by the coated antibody. Following extensive washing, a c-Myc mouse mAb is added to detect the captured c-Myc protein. Anti-mouse IgG, HRP-linked antibody is then used to recognize the bound detection antibody. Chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of c-Myc protein.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: PathScan® Total c-Myc Chemiluminescent Sandwich ELISA Kit #45449 detects endogenous levels of c-Myc in human or mouse cells. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background: Members of the Myc/Max/Mad network function as transcriptional regulators with roles in various aspects of cell behavior including proliferation, differentiation and apoptosis (1). These proteins share a common basic-helix-loop-helix leucine zipper (bHLH-ZIP) motif required for dimerization and DNA-binding. Max was originally discovered based on its ability to associate with c-Myc and found to be required for the ability of Myc to bind DNA and activate transcription (2). Subsequently, Max has been viewed as a central component of the transcriptional network, forming homodimers as well as heterodimers with other members of the Myc and Mad families (1). The association between Max and either Myc or Mad can have opposing effects on transcriptional regulation and cell behavior (1). The Mad family consists of four related proteins; Mad1, Mad2 (Mxi1), Mad3 and Mad4, and the more distantly related members of the bHLH-ZIP family, Mnt and Mga. Like Myc, the Mad proteins are tightly regulated with short half-lives. In general, Mad family members interfere with Myc-mediated processes such as proliferation, transformation and prevention of apoptosis by inhibiting transcription (3,4).

Background References:

- (1) Baudino, T.A. and Cleveland, J.L. (2001) *Mol Cell Biol* 21, 691-702.
- (2) Blackwood, E.M. and Eisenman, R.N. (1991) *Science* 251, 1211-7.
- (3) Henriksson, M. and Lüscher, B. (1996) *Adv Cancer Res* 68, 109-82.
- (4) Grandori, C. et al. (2000) *Annu Rev Cell Dev Biol* 16, 653-99.

| Products Included | Volume | Solution Color |
|--|----------|---------------------|
| c-Myc Rabbit mAb Coated Microwells* | 96 tests | |
| c-Myc Mouse Detection mAb | 1 each | Green (lyophilized) |
| Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated) | 1 each | Red (lyophilized) |
| Detection Antibody Diluent | 5.5 ml | Green |
| HRP Diluent | 5.5 ml | Red |
| Luminol/Enhancer Solution | 3 ml | Colorless |
| Stable Peroxide Buffer | 3 ml | Colorless |
| Sealing Tape | 2 sheets | |
| 20X Wash Buffer | 25 ml | Colorless |
| ELISA Sample Diluent | 25 ml | Blue |
| 10X Cell Lysis Buffer #9803** | 15 ml | Yellowish |

* 12 8-well modules -Each module is designed to break apart for 8 tests.

**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

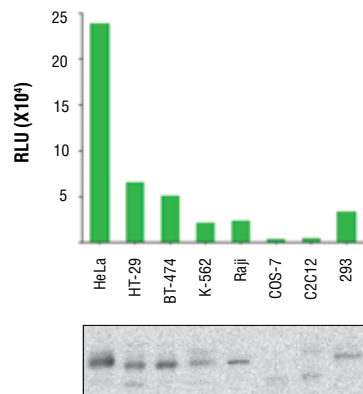


Figure 1. Detection of c-Myc protein is examined across multiple cell lines using the PathScan® Total c-Myc Chemiluminescent Sandwich ELISA Kit. Immediate light generation with chemiluminescent substrate is shown in the top figure, while the corresponding western blot using c-Myc (D3N8F) Rabbit mAb #13987 shown in the bottom figure.

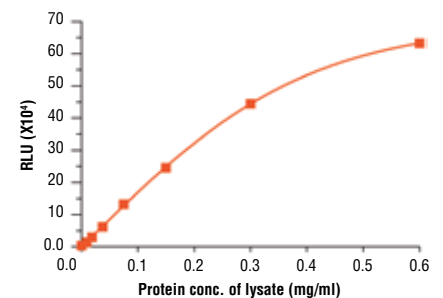


Figure 2. The relationship between protein concentration of cell lysate and immediate light generation with chemiluminescent substrate as detected by the PathScan® Total c-Myc Chemiluminescent Sandwich ELISA Kit is shown. HeLa cells (85% confluent) were harvested and then lysed with the Cell Lysis Buffer (#9803).

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

PathScan® Chemiluminescent Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

NOTE: Refer to product-specific datasheets for assay incubation temperature. This chemiluminescent ELISA is offered in low volume microplates. Only 50 µl of samples or reagents are required in each microwell.

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

- 1. Microwell strips:** Bring all to room temperature before use.
- 2. Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 0.5 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 0.5 ml volume of reconstituted Detection Antibody to 5.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- 3. HRP-Linked Antibody*:** Supplied lyophilized as a red colored cake or powder. Add 0.5 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 0.5 ml volume of reconstituted HRP-Linked Antibody to 5.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- 4. Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (5.5 ml provided).
- 5. HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (5.5 ml provided).
- 6. Sample Diluent:** Blue colored diluent for dilution of cell lysates.
- 7. 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- 8. Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethyl-sulfonyl fluoride (PMSF) immediately before use.
- 9. Luminol/Enhancer Solution and Stable Peroxide Buffer**

*Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.

B Preparing Cell Lysates

For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 × 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
3. Add 50 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at room temperature. Alternatively, the plate can be incubated overnight at 4°C.
4. Gently remove the tape and wash wells:
 - a. Discard plate contents into a receptacle.
 - b. Wash 4 times with 1X Wash Buffer, 150 µl each time for each well.
 - c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
 - d. Clean the underside of all wells with a lint-free tissue.
5. Add 50 µl of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at room temperature for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 50 µl of reconstituted HRP-linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate at room temperature for 30 min.
8. Repeat wash procedure (Section C, Step 4).
9. Prepare Detection Reagent Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50 µl of the Detection Reagent Working Solution to each well.
11. Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nm within 1–10 min following addition of the substrate. *Optimal signal intensity is achieved when read within 10 min.*