Revision 2

Store at +4C

#45449

PathScan® Total c-Myc Chemiluminescent Sandwich ELISA Kit

Cell Signaling TECHNOLOGY®

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Species Cross Reactivity: H

UniProt ID: #P01106

Entrez-Gene Id: #4609

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Quantity	Color	Storage Temp
c-Myc Mouse Detection mAb	76988	1 ea	Green (Lyophilized)	+4C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 ea	Red (Lyophilized)	+4C
Detection Antibody Diluent	13339	5.5 ml	Green	+4C
HRP Diluent	13515	5.5 ml	Red	+4C
Luminol/Enhancer Solution	84850	3 ml	Colorless	RT
Stable Peroxide Buffer	42552	3 ml	Colorless	RT
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	+4C
ELISA Sample Diluent	11083	25 ml	Blue	+4C
Sealing Tape	54503	2 ea		+4C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-20C

Kit contents scale proportionally with size, except sealing tape.
Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

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 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.

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PathScan® Total c-Myc Chemiluminescent Sandwich ELISA Kit

ELISA Chemiluminescent (Lyophilized)

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 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 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. **HRP Diluent**: Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody.
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 phenylmethylsulfonyl 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 (x14,000 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 x 10^6 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 (x14,000 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:
    1. Discard plate contents into a receptacle.
    2. Wash 4 times with 1X Wash Buffer, 150 µl each time for each well.
    3. 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.
    4. 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 Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
10. Add 50 µl of the 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.

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