Store at 4°C

#12946

PathScan® Phospho-M-CSF Receptor (Tyr699) Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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

Species Cross-Reactivity: H

Description: The PathScan® Phospho-M-CSF Receptor (Tyr699) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of M-CSF receptor protein phosphorylated at Tyr699. A M-CSF receptor mouse mAb has been coated onto the microwells. After incubation with cell lysates, both phospho- and nonphospho-M-CSF receptor proteins are captured by the coated antibody. Following extensive washing, Phospho-M-CSF Receptor (Tyr699) Rabbit Detection mAb is added to detect the captured phospho-M-CSF receptor proteins. Anti-rabbit IgG, HRP-linked antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of M-CSF receptor protein phosphorylated at Tyr699.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: PathScan® Phospho-M-CSF Receptor (Tyr699) Sandwich ELISA Kit detects endogenous levels of M-CSF receptor protein phosphorylated at Tyr699 in human cells, as shown in Figure 1. The kit sensitivity is shown in Figure 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background: Macrophage-colony stimulating factor (M-CSF, CSF-1) receptor is an integral membrane tyrosine kinase encoded by the *c-fms* proto-oncogene. M-CSF receptor is expressed in monocytes (macrophages and their progenitors) and drives growth and development of this blood cell lineage. (1-3). Binding of M-CSF to its receptor induces receptor dimerization, activation, and autophosphorylation of cytoplasmic tyrosine residues used as docking sites for SH2-containing signaling proteins (4). There are at least five major tyrosine autophosphorylation sites. Tyr723 (Tyr721 in mouse) is located in the kinase insert (KI) region. Phosphorylated Tyr723 binds the p85 subunit of PI3 kinase as well as PLCγ2 (5). Phosphorylation of Tyr809 provides a docking site for Shc (5). Overactivation of this receptor can lead to a malignant phenotype in various cell systems (6). The activated M-CSF receptor has been shown to be a predictor of poor outcome in advanced epithelial ovarian carcinoma (7) and breast cancer (8).

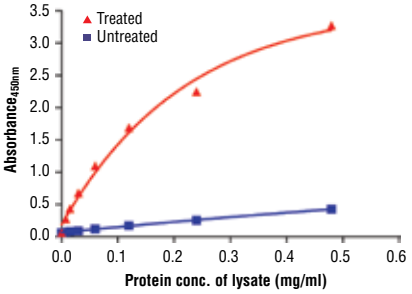
Phosphorylation of M-CSF receptor at Tyr699 was identified at Cell Signaling Technology (CST) using PhosphoScan®, CST's LC-MS/MS platform for phosphorylation site discovery, and was cited in another publication (10). Autophosphorylation at Tyr699 within the kinase insert (KI) domain appears to provide a binding site for the Grb2 adaptor protein (9).

Product Includes	Item #	Kit Quantity	Color	Storage Temp.
M-CSF Receptor Mouse mAb Coated Microwells*	27190	96 tests		4°C
Phospho-M-CSF Receptor (Tyr699) Rabbit Detection mAb	62330	1 each	Green (Lyophilized)	4°C
Anti-rabbit IgG, HRP-linked Antibody	13272	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml		-20°C

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

Background References:

(1) Stanley, E.R. et al. (1978) *Nature* 274, 168-70.
(2) Byrne, P.V. et al. (1981) *J Cell Biol* 91, 848-53.
(3) Bourette, R.P. and Rohrschneider, L.R. (2000) *Growth Factors* 17, 155-66.
(4) Novak, U. et al. (1996) *Oncogene* 13, 2607-13.
(5) Bourette, R.P. et al. (1997) *EMBO J* 16, 5880-93.
(6) Morley, G.M. et al. (1999) *Oncogene* 18, 3076-84.
(7) Toy, E.P. et al. (2001) *Gynecol Oncol* 80, 194-200.
(8) Maher, M.G. et al. (1998) *Clin Cancer Res* 4, 1851-6.
(9) Hamilton, J.A. (1997) *J Leukoc Biol* 62, 145-55.
(10) Downing, J.R. et al. (1991) *Mol Cell Biol* 11, 2489-95.



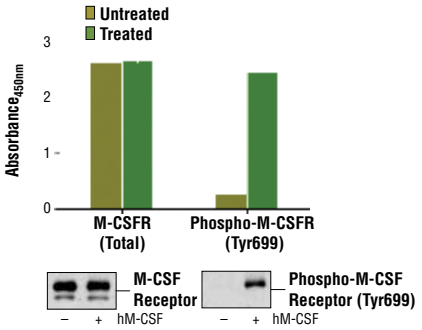


Figure 1: Treatment of NKM-1 cells with Human Macrophage Colony Stimulating Factor (hM-CSF) #8929 stimulates tyrosine phosphorylation of M-CSF receptor protein, as detected by PathScan® Phospho-M-CSF Receptor (Tyr699) Sandwich ELISA Kit, but does not affect the level of total M-CSF receptor detected by PathScan® Total M-CSF Receptor Sandwich ELISA Kit #13032. The absorbance readings at 450 nm are shown in the top figure, while corresponding western blots using M-CSF Receptor Antibody #3152 (left panel) and Phospho-M-CSF Receptor (Tyr699) Antibody #12251 (right panel) are shown in the bottom figure.

◀ *Figure 2: The relationship between protein concentration of lysates from untreated and hM-CSF-treated NKM-1 cells and the absorbance at 450 nm as detected by the PathScan® Phospho-M-CSF Receptor (Tyr699) Sandwich ELISA Kit is shown. Unstarved NKM-1 cells (0.5-1.0 x 10^6) were treated with Human Macrophage Colony Stimulating Factor (hM-CSF) #8929 (50 ng/ml, 2-5 min, 37°C) and then lysed.*

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **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.

#12946

PathScan® Sandwich ELISA (Colorimetric)

A. Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
3. **1X 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.
4. **1X PBS:** (20X Phosphate Buffered Saline #9808).
5. **TMB Substrate** (#7004)
6. **STOP Solution** (#7002)

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 minutes.
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 information regarding an appropriate dilution factor for lysates and kit assay results.
3. Add 100 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 hours at 37°C. 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, 200 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 100 µl of Detection Antibody (green color) to each well. Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of HRP-Linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
10. Add 100 µl of STOP Solution to each well. Shake gently for a few seconds.

NOTE: Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

1. **Read results**
   a. **Visual Determination:** Read within 30 min after adding STOP Solution.
   b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.