p53 Total and p53 (Phospho-Ser^{392}) Dual Staining Assay Kit

Item No. 600060

Customer Service 800.364.9897 * **Technical Support** 888.526.5351

www.caymanchem.com

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	100 Tests Quantity	Storage
10009899	Cell-Based Assay Fixative	1 vial	Room Temperature
600033	Cell-Based Assay p53 (Phospho-Ser^{392}) Polyclonal Primary Antibody	1 vial	-20°C
10009906	Cell-Based Assay Blocking Solution	1 vial	4°C
10011231	DyLight™ 488-Conjugated Goat Anti-Rabbit Secondary Antibody	1 vial	-20°C
600061	Cell-Based Assay p53 Total Monoclonal Primary Antibody	1 vial	4°C
600062	DyLight™ 549-Conjugated Goat Anti-Mouse Secondary Antibody	1 vial	-20°C
600034	Cell-Based Assay (-)-Nutlin-3 (10 mM)	1 vial	-20°C

NOTE: DyLight™ 488 and 549 are products of Thermo Fisher Scientific Inc.

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A 6-, 12-, 24-, or 96-well plate
2. MCF-7 cells (can be obtained from ATCC); other cell lines can also be used
3. Immunocytochemical staining buffer, TBS, pH 7.4
4. Triton-X 100
5. A fluorescence microscope equipped with filters capable of excitation/emission pairs at 485/535 nm and 550/568 nm

INTRODUCTION

Background

p53 is a tumor suppressor protein that is commonly referred to as the "guardian of the genome" because of its crucial role in coordinating cellular responses to genotoxic stress.[1] The tumor suppressor activity of p53 is mediated by a variety of mechanisms including cell cycle arrest, apoptosis, and cellular senescence. Approximately 50% of human cancers carry a mutation in the p53 gene; of those tumors that do not have a mutation in the p53 gene, a significant proportion of them have inactivated p53 by alternative mechanisms.[2] These characteristics make p53 a useful biomarker in carcinogenesis. p53 has important clinical implications in the treatment of cancer and is a focus of cancer drug discovery. The regulation of p53 levels and activity involves a complex network of cellular proteins including HPV16, PARP-1, WT1, E1b/E4, Mdm2, and others. WT1 or E1B/E4 bind to p53 increasing its stability whereas p53's binding with Mdm2 accelerates its degradation through ubiquitination and subsequent degradation.[3] The Mdm2 gene contains a p53 promoter and is therefore transcriptionally regulated by p53 during stress. In this manner p53 itself regulates Mdm2 at the level of transcription, whereas Mdm2 regulates p53 protein activity.[4]

Under normal cellular conditions, p53 is maintained at low concentrations and/or in an inactive form. Upon stimulation by environmental stress, p53 can be activated through: an increase of the p53 protein concentration due to an increase in translation or protein half-life, transformation of the protein from an inactive to an active conformation, or the phosphorylation/translocation of the p53 protein from the cytoplasm to the nucleus.[5] Although there are a number of studies investigating the modulation of p53 activity by the first two mechanisms, few researchers have addressed the regulation of p53 subcellular localization.

About This Assay

Cayman's p53 Total and p53 (Phospho-Ser^{392}) Dual Staining Assay Kit provides a pair of highly specific antibodies against total p53 (both un-phosphorylated and phosphorylated) and phospho-p53 (phospho-Ser^{392}) together with a pair of matched DyLight™ (product of Thermo Fisher Scientific Inc.) conjugated secondary antibodies in a ready-to-use format. (-)-Nutlin-3, a potent inhibitor of the Mdm2-p53 interaction, which has been shown by scientists at Cayman to cause the p53 activation and translocation of phosphorylated p53 (phospho-Ser^{392}) between the cytoplasm and nuclear compartments, is included as a positive control.

ASSAY PROTOCOL

Treatment of Cells

The following protocol is designed for a 96-well plate. For other sizes of plates the volume of medium/solution to apply to each well should be adjusted accordingly.

1. Seed a 96-well plate with 3 x 10^4 cells/well; grow cells one day or until 80% confluent.
2. The next day, treat cells with experimental compounds or vehicle for four hours, or for the period of time required for your typical experimental protocol. To use the included (-)-Nutlin-3 as a positive control, dilute the Cell-Based Assay (-)-Nutlin-3 (10 mM) (Item No. 600034) 1:200 into your culture medium.
3. Terminate the experiment and examine sub-cellular localization of total p53 and phospho-Ser^{392}-p53 using the following immunocytochemical staining procedure.

Immunofluorescence Staining Procedure

Perform all steps at room temperature. For a 96-well plate, the assay volume for each well is 100 µl. For other plate sizes, the volume of reagent to apply to each well should be adjusted accordingly. We recommend that each treatment is performed in triplicate. We suggest you record the contents of each well on the template sheet provided (see page 11).

1. Remove most of the medium from the wells.
2. Wash cells briefly with TBS, pH 7.4.
3. Fix the cells with Cell-Based Assay Fixative (Item No. 10009899) for 15 minutes.
4. Wash wells with TBS containing 0.1% Triton-X 100 (TBST) three times for five minutes each.
5. Incubate the cells with Cell-Based Assay Blocking Solution (Item No. 10009906) for 30 minutes (go to step 6 directly without performing a wash step).
6. Incubate the cells for two hours with 100 µl of a cocktail solution of primary antibodies (Item No. 600033 and 600061) diluted 1:200 in TBST. Alternatively, incubate the cells in the antibody cocktail overnight at 4ºC.
7. Wash the cells three times with TBST for five minutes each.
8. Incubate the cells in the dark for one hour with 100 µl of a cocktail solution of both secondary antibodies (Item No. 10011231 and 600062) diluted 1:100 in TBST.
9. Wash the cells three times with TBST for five minutes each.
10. Examine the total p53 staining using a microscope with filters capable of excitation and emission at 550 and 565nm, respectively; examine the p53 (phospho-Ser^{392}) with filters capable of excitation and emission at 485 and 535 nm, respectively. Alternatively, store the plate at 4ºC in the dark for later analysis. The staining is stable for up to three days at 4ºC.

PERFORMANCE CHARACTERISTICS

Cell Staining

NOTE: The results below were obtained with culture conditions as described in the protocol. Your results may not necessarily be identical to this, as the response of the protein may vary greatly depending on cell type, chemical compound dose, treatment duration, cell density, and culture conditions.

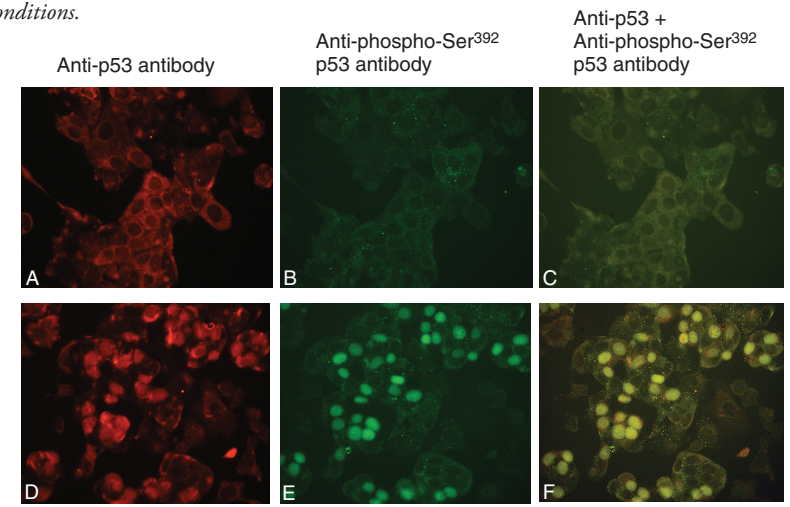


Figure 1. (-)-Nutlin-3-induced translocation of p53 in MCF-7 cells. *Top panels:* MCF-7 cells were treated with vehicle. *Bottom panels:* 50 µM (-)-Nutlin-3 after four hours, then fixed and stained as described in the assay protocol. *Panel A and B:* show that in unstimulated MCF-7 cells, most of p53 was not phosphorylated and appeared as cytoplasmic staining (strong staining of total protein in A and weak staining of phosphorylated protein in B). *Panel C:* is the merged image of A and B. *Panel D and E:* in contrast show that upon stimulation by (-)-Nutlin-3, most of p53 was phosphorylated and appeared in the nucleus (strong staining of both total protein and phosphorylated protein in Both D and E, respectively). *Panel F:* is the merged image of D and E.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No signal in all wells	Omission of key reagent	Check that all reagents have been added and in the correct order
No response following treatment	Cells are too old	Use lower passage cells

References

1. Brooks, C.L. and Gu, W. p53 ubiquitination: Mdm2 and beyond. *Molecular Cell* **21**, 307-315 (2006).
2. Haupt, S., Berger, M., Goldberg, Z., *et al.* Apoptosis-the p53 network. *J. Cell Sci.* **116**, 4077-4085 (2003).
3. Gasco, M., Shami, S., and Crook, T. The p53 pathway in breast cancer. *Breast Cancer Research* **4**, 70-76 (2002).
4. Dey, A., Verma, C.S., and Lane, D.P. Updates on p53: Modulation of p53 degradation as a therapeutic approach. *Br. J. Cancer* **98**, 4-8 (2008).
5. Liang, S.-H. and Clarke, M.F. Regulation of p53 localization. *Eur. J. Biochem* **268**, 2779-2783 (2001).

Related Products

p53 Cell-Based Activation/Translocation Assay Kit - Item No. 600008
p53 Transcription Factor Assay Kit - Item No. 600020
p53 Designer Transcription Factor Assay Kit - Item No. 600030

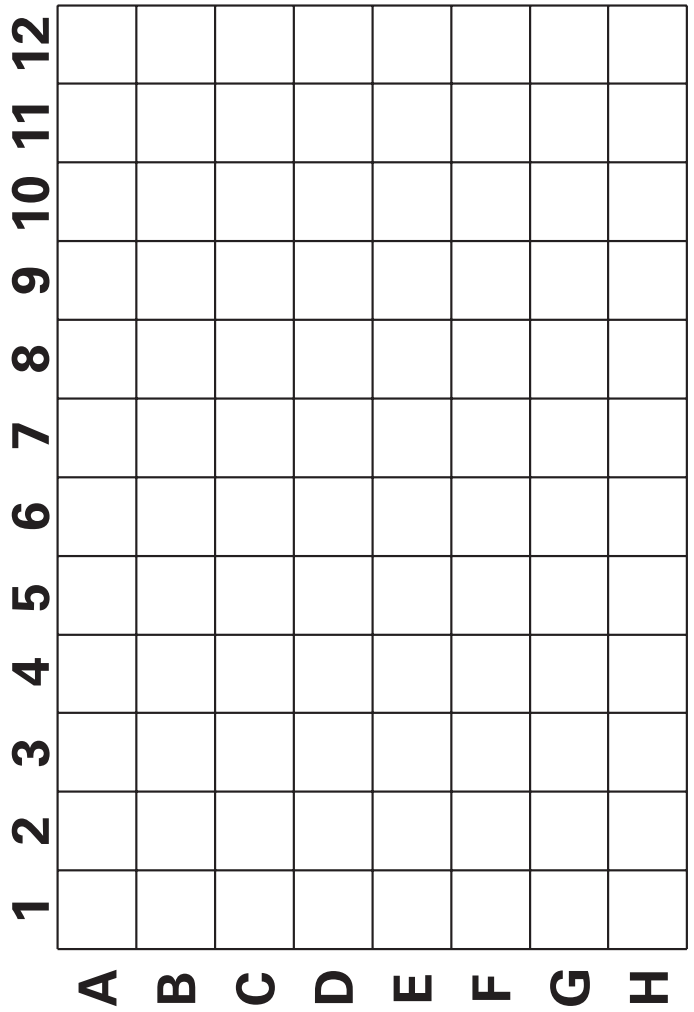
Warranty and Limitation of Remedy

Cayman Chemical Company makes **no warranty or guarantee** of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman **warrants only** to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have **any obligation or liability**, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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