p53 Cell-Based Activation/Translocation Assay Kit
Item No. 600008
Materials Supplied

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

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>100 Tests Quantity</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>10009899</td>
<td>Cell-Based Assay Fixative</td>
<td>1 vial</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>600061</td>
<td>Cell-Based Assay p53 Total Monoclonal Primary Antibody</td>
<td>1 vial</td>
<td>4°C</td>
</tr>
<tr>
<td>10009906</td>
<td>Cell-Based Assay Blocking Solution</td>
<td>1 vial</td>
<td>4°C</td>
</tr>
<tr>
<td>600062</td>
<td>DyLight™ 549-Conjugated Goat Anti-Mouse Secondary Antibody</td>
<td>1 vial</td>
<td>-20°C</td>
</tr>
<tr>
<td>600034</td>
<td>Cell-Based Assay (-)-Nutlin-3 (10 mM)</td>
<td>1 vial</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

NOTE: DyLight™ 549 is a product 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, on page 3, 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 and emission at 549 and 568 nm, respectively.

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. 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. 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. 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, where Mdm2 protein regulates p53 protein activity.

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 elongated protein half-life, transformation of the protein from an inactive to an active conformation, or the activation/translocation of the p53 protein from the cytoplasm to the nucleus. 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 Cell-Based Activation/Translocation Assay Kit provides a highly specific p53 primary monoclonal antibody together with a DyLight™ (product of Thermo Fisher Scientific Inc.) conjugated secondary antibody in a ready-to-use format. (-)-Nutlin-3, a potent inhibitor of Mdm2-p53 interaction which has been shown by scientists at Cayman to cause the activation and translocation of p53 between the cytoplasm and nuclear compartments, is included as a positive control.
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 $3 \times 10^4$ 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 subcellular localization of p53 using the following immunocytochemical staining procedure.

Immunofluorescence Staining Procedure

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 15).

1. Remove most of the medium from the wells.
2. Wash cells briefly with TBS, pH 7.4.
3. Fix the cell 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 with the Cell-Based Assay p53 Monoclonal Primary Antibody (Item No. 600061) diluted 1:200 in TBST for two hours. Alternatively, incubate the cells with the Primary Antibody overnight at 4°C.
7. Wash the cells three times with TBST for five minutes each.
8. Incubate the cells in the dark for one hour with the DyLight™ 549-Conjugated Goat Anti-Mouse Secondary Antibody (Item No. 600062) diluted 1:100 in TBST.
9. Wash the cells three times with TBST for five minutes each.
10. Examine the fluorescent staining using a microscope with filters capable of excitation and emission at 550 and 568 nm, respectively. Alternatively, store the plate at 4°C in the dark for later analysis. The staining is stable for up to three days at 4°C.
**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 types, chemical compound dose, treatment duration, cell density, and culture conditions.

![Image A]

![Image B]

Figure 1. (-)-Nutlin-3-induced translocation of p53 in MCF-7 cells. Panel A: MCF-7 cells were treated with vehicle or Panel B: 50 µM (-)-Nutlin-3 for four hours, then fixed and stained with p53 monoclonal antibody according to the protocol described in the booklet. Translocation of p53 from cytoplasm to nuclei upon stimulation by (-)-Nutlin-3 is evident.

**Troubleshooting**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signal in all wells</td>
<td>Omission of key reagent</td>
<td>Check that all reagents have been added and in the correct order</td>
</tr>
<tr>
<td>No response following treatment</td>
<td>Cells are too old</td>
<td>Use lower passage cells</td>
</tr>
</tbody>
</table>

**References**


**Related Products**

- p53 Transcription Factor Assay Kit - Item No. 600020
- p53 Designer Transcription Factor Assay Kit - Item No. 600030
- p53 Total and p53 (Phospho-Ser539) Dual Staining Assay Kit - Item No. 600060

**RESOURCES**
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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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