p53 Nuclear Translocation Assay Kit (Cell-Based) (#BN01121)

(Catalog # BN01121; 50 assays; Store at -20°C)

I. Introduction:

p53 is a tumor suppressor gene which encodes a transcription factor that controls cell's destiny. This gene gets activated when cells are exposed to increased cellular stress, or DNA damage. The levels of p53 are elevated when post-translational modifications occur, which block its sequestration and/or ubiquitination by its destabilizer, Mouse Double Minute 2 Homolog (MDM2) which in turn is a transcriptional target of p53. Due to the three putative nuclear localization signals (NLSs) on its C-terminus, some of the activated p53 protein translocates into the nucleus and activate genes that induce cell cycle arrest, senescence, or apoptosis. The p53 gene is frequently mutated in cancer cells, and about 50% of cancers show p53 mutations, resulting in loss of its functions. The loss of p53 functionality can lead to dysregulation of many biological processes in cells, such as metabolic pathways, cellular homeostasis, cell movement, apoptosis, etc. Assay Genie's p53 Nuclear Translocation Assay Kit (Cell-Based) provides an easy and complete assay kit to visualize the activation and nuclear translocation of p53 in human cells. This assay kit uses Assay Genie's specific and sensitive human p53 antibody and a p53 secondary antibody to visualize the localization of p53 in fixed common human cells along with DAPI, a fluorescent stain, for nuclear staining. The kit includes Nutlin-3, a potent selective inhibitor that disrupts the protein-protein interaction between p53 and MDM2. Nutlin-3 serves as a control to induce p53 translocation from the cytoplasm to the nucleus.

II. Applications:

- Visualize nuclear translocation and activation of p53 protein in mammalian cells
- Detection of increased cellular stress or DNA damage
- Screening for potential inhibitors that disrupt the interaction between p53 and MDM2

III. Sample Type:

- Adherent or suspension human cells

IV. Kit Contents:

Components	BN01121	Cap Code	Part Number
1X Fixative Solution	15 ml	WM	BN01121-1
1X Permeabilization Buffer	15 ml	NM	BN01121-2
1X Blocking Buffer	40 ml	NM	BN01121-3
p53 Primary Antibody (500X)	30 µl	Brown	BN01121-4
p53 Secondary Antibody (500X)	30 µl	Red	BN01121-5
Nutlin-3 Reagent (200X)	30 µl	Yellow	BN01121-6
DAPI (1000X)	20 µl	Blue	BN01121-7

V. User Supplied Reagents and Equipment:

- 24 or 48-well clear bottom tissue culture plate
- Phosphate Buffered Saline (PBS)
- Shaker
- 0.1% Gelatin Solution (optional, only required for Suspension Cells)
- Fluorescence microscope (550 nm excitation and UV filter for DAPI)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. All components are stable for at least 1 year. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **1X Fixative Solution, 1X Permeabilization Buffer and 1X Blocking Buffe**r: Store them at 4°C.
- **p53 Primary Antibody, p53 Secondary Antibody**: Keep on ice while in use. Aliquot and store at -20°C. Avoid freeze/thaw cycle.
- **Nutlin-3:** Thaw it before use. Store at -20°C.
- **DAPI:** Store them at 4°C.

VII. p53 Nuclear Translocation Assay Protocol:

1. Sample Preparation:

Adherent cells: Seed cells (1-2 x 10^4/well) in a 48-well tissue culture plate, and add complete medium to a final volume of 250 µl/well the day before starting the experiment to allow cell attachment.

Suspension cells: Add 250 µl of 0.1% gelatin solution into each well in a 48- well tissue culture plate, tilt the plate, in order for the gelatin solution to cover the entire surface. Place it in a culture hood for 1 h. and remove the 0.1% gelatin solution. Seed cells (1-2 x 10^4/well) in the previously made gelatin coated 48-well tissue culture plate, and add medium, supplemented with FBS, to a final volume of 250 µl/well the day before starting the experiment to allow cell attachment.

The following day, treat cells with or without test compound(s). Incubation time is based on the test compounds (e.g. 4-12 hrs treatment). As a positive control, Nutlin-3 Reagent (200X) can be used to treat cells for 4 hrs to induce p53 translocation. For every positive control: add 1.25 µl of stock Nutilin-3 reagent, and make up volume to 250 µl with complete medium. Mix well.

Notes:

All volumes in this protocol are calculated based on using a 48-well tissue culture plate. For different size Tissue Culture plate, adjust the volume so that volume of liquid can fully cover the bottom of wells.

2. **Cell Fixation**: When treatment is complete, remove medium, and gently wash cells once with 250 µl of PBS without shaking. Fix cells with 250 µl/well of 1X Fixative Solution for 20 min at room temperature.
3. **Permeabilization and Blocking:** Remove 1X Fixative Solution, and wash twice with 250 µl of PBS for 5 min each on a shaker. Add 250 µl of 1X Permeabilization Buffer and incubate for 20 min at room temperature on a shaker. Remove 1X Permeabilization Buffer and wash cells once with 250 µl of PBS. Remove PBS and add 250 µl of 1X Blocking Buffer to each well and incubate for 20 min at room temperature on a shaker.
4. **First Antibody Incubation:** During blocking, dilute p53 primary antibody in 1X Blocking Buffer at a dilution factor of 1:500 (e.g. Add 2 µl of p53 secondary antibody to 998 µl 1X Blocking Buffer, mix well). After blocking is complete, remove 1X Blocking Buffer, and add 250 µl of diluted p53 primary antibody to each well. Incubate cells in p53 primary antibody for 1 hr at room temperature or at 4°C overnight.
5. **Second Antibody Incubation:** After p53 primary antibody incubation, wash cells three times with 250 µl of PBS on a shaker for 5 min each. During washing, dilute p53 secondary antibody in 1X Blocking Buffer at a dilution factor of 1:500 (e.g. Add 2 µl of p53 secondary antibody to 998 µl 1X Blocking Buffer, mix well). Add 250 µl of diluted p53 secondary antibody to each well, and incubate for 1 hour at room temperature.
6. **DAPI Staining:** After secondary antibody staining, wash cells in 250 µl of PBS two times on a shaker for 5 min each. During washing, prepare 250 µl of 1X DAPI staining buffer for each well. Dilute stock DAPI (1000X) in PBS at 1:1000 dilution factor. After the second wash, add 250 µl of 1X DAPI staining buffer to each well, cover with foil to protect from light and incubate for 10 min at room temperature on a shaker. After DAPI staining, remove 1X DAPI staining buffer, wash 1 time with 250 µl PBS and add 250 µl of fresh PBS. Cells are ready to be imaged under a fluorescent microscope with 550 nm excitation laser (for p53) and UV laser (for DAPI)

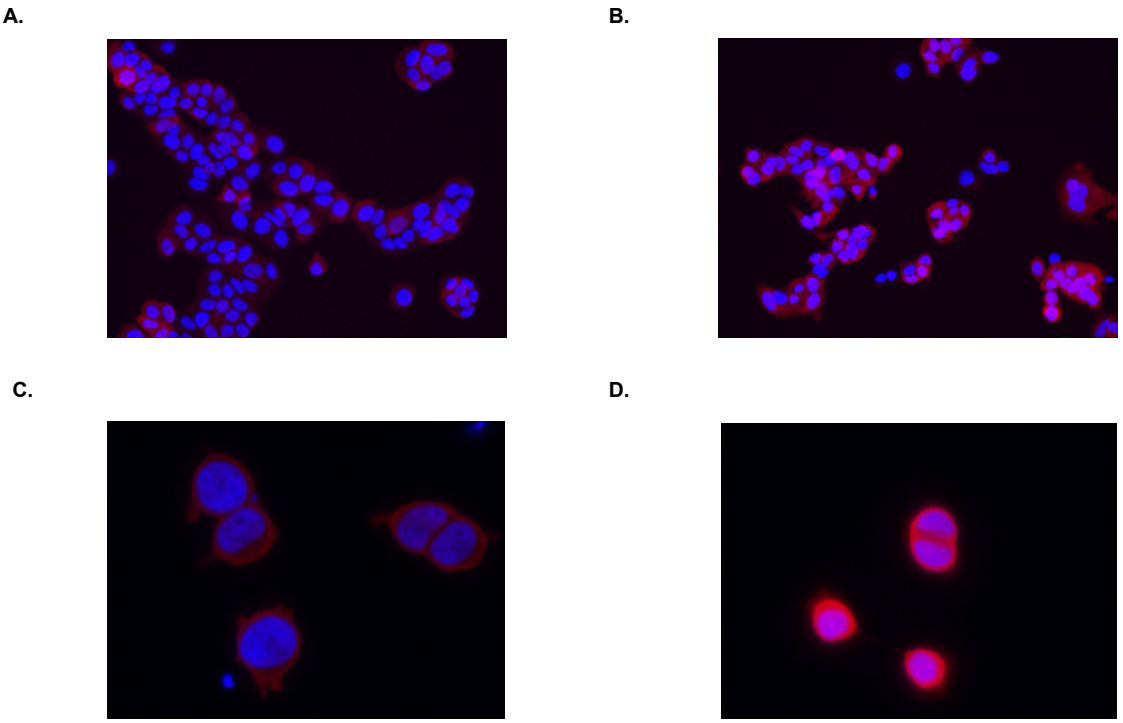

Figure: p53 Activation and Translocation in MCF-7 cells. MCF-7 cells were seeded into a 48-well tissue culture plate at $1X10^4$ cells/well. After 1 day of culturing, cells were treated with 1X Nutlin-3 for 4 hrs. After treatment, cells were fixed and stained for p53 translocation and DAPI according to the kit's protocol. **A&C**: MCF-7 cells were treated with vehicle control; **B&D**: MCF-7 cells treated with 1X Nutlin-3. (Magnification used **A&B**: 20X; **C&D**: 60X oil).

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