ab284563 – p53 Nuclear Translocation Assay Kit (Cell-Based)

For the visualization of nuclear translocation of p53 in mammalian cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284563

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
1X Blocking Buffer	40 mL	+4°C
1X Fixative Solution	15 mL	+4°C
1X Permeabilization Buffer	15 mL	+4°C
DAPI (1000X)	20 µl	+4°C
Nutline-3 Reagent (200X)	30 µl	-20°C
p53 Primary Antibody (500X)	30 µl	-20°C
P53 Secondary Antibody (500X)	30 µl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

1X Fixative Solution, 1X Permeabilization Buffer and 1X Blocking Buffer: 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 at 4°C.

Assay Protocol

1. Sample preparation:

***Δ Note**: 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.*

a) **Adherent cells:** Seed cells (1-2 x 104 /well) in a 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.

b) **Suspension cells:** Add 250 µl of 0.1% gelatin solution into each well in a 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 104 /well) in the previously made gelatin coated 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.

c) 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).

d) 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.

2. Permeabilization and Blocking:

a) 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. Remove 1X Fixative Solution and wash twice with 250 µl of PBS for 5 min each on a shaker.

b) 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.

3. Immunofluorescence Staining:

Δ Notes: The recommended dilution for primary and secondary antibodies is 1:500 but it may vary for different cell lines. During the incubation the plate should always be covered and protected from light to prevent drying and photobleaching.

a) **Primary Antibody Incubation:** During blocking, dilute p53 primary antibody in 1X Blocking Buffer at a dilution factor of 1:500. 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.

b) **Secondary 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. Add 250 µl of diluted p53 secondary antibody to each well and incubate for 1 hour at room temperature.

c) **DAPI Staining:** After secondary antibody staining, wash cells in 250 µl of PBS two times on a shaker for 5 min each. Dilute DAPI stain 1:1000 in PBS, aliquot 250 µl to each well and incubate for 10 minutes in the dark. Remove the stain and wash 1 time with 250 µl PBS and add 250 µl of fresh PBS.

d) Examine the staining under fluorescence microscope with 550 nm excitation laser for (p53) and UV laser (for DAPI).

Technical Support

For all technical or commercial enquiries please go to:

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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)