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ab252888
TUNEL Assay Kit - Edu-Orange

View kit datasheet: www.abcam.com/ab252888

(use www.abcam.cn/ab252888 for China, or www.abcam.co.jp/ab252888 for Japan)

For the identification of damaged DNA by FACS or fluorescence microscopy.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

TUNEL Assay Kit - Edu-Orange (ab252888) utilizes modified EdUTP nucleotides which are incorporated at the 3'-OH ends of fragmented DNA by Terminal deoxynucleotidyl transferase (TdT) enzyme and detected based on a click reaction.

This assay enables easy delivery and incorporation of EdUTP coupled with high selectivity of detection of DNA damage. The assay is performed under mild reaction conditions which preserve cell morphology, thus enabling the identification of damaged DNA by FACS or fluorescence microscopy.

Our assay is fast and capable of detecting a higher percentage of apoptotic cells than the antibody-based methods.

The kit contains sufficient reagents to detect total/fragmented DNA in apoptotic cells in a 1 X 96-well plate or on 50 cover slips.

2. Protocol Summary

Prepare control and experimental cells.

Add TUNEL reaction cocktail to all wells except background control and un-stained cells. Incubate for 1 hr at 37°C OR overnight at RT.

Add click reaction cocktail to all cells except un-stained cells. Incubate for 30 mins at RT in the dark.

Analyze samples for red fluorescence generated by TUNEL positive cells and green by total DNA respectively (FM).

OR

Analyze samples in FL-2 channel for signal generated by TUNEL positive cells during click reaction (FACS).

3. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
10X Wash Buffer IV/Wash Buffer (10X)	25 mL	-20°C	4°C
Fixative Solution I/Fixative Solution	10 mL	-20°C	4°C
10X Permeabilizationn Buffer/Permeabilization Buffer (10X)	25 mL	-20°C	4°C
50X EdUTP DNA Label/EdUTP DNA Label (50X)	100 µL	-20°C	-20°C
100X Copper Reagent/Copper Reagent (100X)	100 µL	-20°C	-20°C
100X Fluorescent Azide I/Fluorescent Azide (100X)	100 µL	-20°C	-20°C
20X Reducing Agent/Reducing Agent (20X)	500 µL	-20°C	-20°C
1000X Total DNA Stain/Total DNA Stain (1000X)	10 µL	-20°C	-20°C
10X TUNEL Reaction Buffer/TUNEL Reaction Buffer (10X)	1 mL	-20°C	-20°C
TUNEL Enzyme	1 vial	-20°C	-20°C
TUNEL Enzyme Buffer	500 µL	-20°C	-20°C

4. Materials Required, Not Supplied

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

- Fluorescence microscope capable of excitation and emission at 440/490 and 540/580 nm, respectively.
- Flow cytometer equipped with laser capable of excitation at 488 and 530/590 nm emission filters, respectively.
- Optional: Sterile 0.1% Gelatin Solution (optional, only required for adhering suspension cells to the surface).
- Tissue culture vessels and appropriate culturing media; flow cytometry vessels.
- Phosphate Buffered Saline (PBS, pH 7.4).

5. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

6. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

6.1 10X Wash Buffer IV/Wash Buffer (10X)

Thaw at 37°C to dissolve completely. Dilute the 10X stock 1:10 in sterile water, mix well. Store at 4°C. Warm to room temperature prior to use.

6.2 Fixative Solution I/Fixative Solution

Divide into aliquots and store at -20°C, protected from light.

6.3 10X Permeabilization Buffer/Permeabilization Buffer (10X)

Thaw at 37°C to dissolve completely. Dilute the 10X stock 1:10 in sterile water, mix well. Store at 4°C. Warm to room temperature prior to use.

6.4 50X EdUTP DNA Label/EdUTP DNA Label (50X)

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.5 100X Copper Reagent/Copper Reagent (100X)

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.6 100X Fluorescent Azide I/Fluorescent Azide (100X)

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.7 Reducing Reagent (20X)

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.8 1000X Total DNA Stain/Total DNA Stain (1000X)

Ready to use as supplied. Store at -20°C protected from light. While in use, keep on ice and minimize light exposure.

6.9 TUNEL Enzyme

Resuspend into 300 µL of TUNEL Enzyme Buffer, mix well. Aliquot and store at -20°C. Use within two months.

7. Assay Procedure

General sample information:

This assay was developed with HeLa (adherent) and Jurkat (suspension) cells and can be modified for any cell line. For this protocol, total assay volume: 100 µL (96 well tissue culture plate). Adjust volumes for other plate formats. Growth conditions, cell number/well etc. should be optimized for your cell type/treatment. We suggest testing several EdUTP DNA Label concentrations to find the best concentration for your experiment. All steps and reagent equilibration should be carried out at room temperature (RT).

7.1 Preparation of control and experimental cells:

1. Obtain cell suspension, and seed directly into tissue culture vessels (HeLa cells (10^5 cells/ ml) and Jurkat (10^6 cells/ ml)), or on coverslips for high resolution microscopy.
2. To immobilize suspension cells for microscopy: add 100 µL of 0.1% gelatin solution directly into the wells, tilt the plate to cover the entire well surface, place it in a tissue culture hood for 1 hour. Gently remove the gelatin solution and seed your cells. Allow the cells to recover overnight before the treatment.
3. Prepare wells with cells that will be treated (Experimental Wells) and 4 controls. **A.** Unstained Cells: (FACS Setup Control Cells-- No TUNEL and No Click); **B.** Positive Control Cells: (DNase-treated cells after step 5. DNAse is not provided); **C.** Background Control Cells: (Click reaction only, no TUNEL); **D.** Negative Control Cells (untreated cells that undergo TUNEL followed by Click reaction).
4. Next day, remove the media and induce apoptosis according to your protocol in the wells with Experimental Cells. For immobilized suspension cells: Centrifuge the plate at 500 *x g* (or the lowest centrifuge setting) for 5 minutes to deposit the cells onto the surface. Tilt the plate and gently to remove the media with a pipette tip. Use the same centrifugation settings throughout the entire protocol.
5. To terminate the experiment for adherent cells, remove culture media, wash the cells once with 100 µL PBS, and then aspirate the PBS. For suspension cells, centrifuge the cells at 500 *x g* for 5 mins. Discard the supernatant and wash the cells once with 100 µL PBS Centrifuge once again at 500 x g for 5 mins, then aspirate the PBS.

7.2 Fixation and Permeabilization:

1. For adherent cells: Add 100 µL of Fixative Solution I/Fixative Solution to each well and incubate the cells for 15 mins at RT protected from light. Remove the fixative and wash the cells once with 100 µL of 1X Wash Buffer IV/Wash Buffer, remove the wash. Add 100 µL of 1X Permeabilization Buffer and incubate the cells for 10 mins at RT. Remove the Permeabilization Buffer and wash the cells twice in 100 µL of dH_2O, discard washes.
2. For suspension cells: Re-suspend the cells in 100 µL of Fixative Solution I/Fixative Solution and incubate for 15 mins at RT protected from light. Centrifuge and remove the fixative and wash the cells once with 100 µL of 1X Wash Buffer IV/Wash Buffer. Centrifuge and remove the supernatant and re-suspend the cells in 100 µL of 1X Permeabilization Buffer. Incubate the cells for 10 mins at RT. Centrifuge and remove the Permeabilization Buffer and wash the cells twice in 100 µL of dH_2O, centrifuge and remove washes each time.
   Δ Note: For Positive Control Cells well(s), treat the cells with 10 U of DNase in 100 µL. Keep the rest of the samples at 4°C in 100 µL of PBS during the DNase treatment of the Positive Control wells.

7.3 TUNEL Reaction:

This protocol uses 50 µL of the TUNEL reaction cocktail per well (100 µL for cover slips). Cells must be equilibrated to maximize the efficiency of TUNEL reaction. **DO NOT** treat the Background Control and Unstained cells.

1. Dilute 10X TUNEL Buffer to 1X with dH_2O and add 50 µL in each well and allow the solution to completely cover the surface. Incubate plates for 10 mins at RT. Spin down at 500 *x g* for 5 mins and remove the TUNEL reaction buffer. Add 50 µL of TUNEL buffer to Background Control and Unstained cells.
2. Prepare 1X TUNEL reaction cocktail according to the table below and add 50 µL to each Experimental, Positive and Negative Control cells well. Incubate the cells for 1 h at 37°C. Alternatively, TUNEL reaction can be carried out overnight at RT. Prepare enough Reaction Cocktail mix for the number of samples that will be analyzed.

Component	Reaction Mix (µL)
dH_2O	41
10X TUNEL Reaction Buffer/TUNEL Reaction Buffer (10X)	5
50X EdUTP DNA Label/EdUTP DNA Label (50X)	1
TUNEL Enzyme	3

3. Spin down at 500 *x g* for 5 mins and remove the reaction cocktail. Add 200 µL of 1X Wash Buffer IV/Wash Buffer, mix well. Spin down at 500 *x g* for 5 mins and remove Wash Buffer IV/Wash Buffer. Re-suspend the cells in 20 µL of 1X Permeabilization Buffer and proceed to click reaction.

7.4 Click reaction and total DNA staining: (Background Control, Negative Control, Positive Control and Experimental cell wells:

1. Reaction cocktail: Prepare 1X click reaction cocktail according to the table below. Reagent should be prepared based on the number of samples to be analyzed. Add reagents in the exact order as indicated below. Use the reaction cocktail within 15 minutes of preparation. Cover the plates with foil to protect from light during and follow the click reaction and DNA staining.

Component	Reaction Mix (µL)
PBS	93
100X Copper Reagent/Copper Reagent (100X)	1
100X Fluorescent Azide I/Fluorescent Azide (100X)	1
20X Reducing Agent/Reducing Agent (20X)	5

2. Click Reaction: Add 100 µL of 1X Click Reaction cocktail to each Experimental, Background control, Positive Control and Negative Control cell wells. Add 100 µL PBS to Unstained Control well(s), Incubate the cells for 30 mins at room temperature protected from light. Centrifuge and remove supernatant and wash cells three times in 100 µL of 1X Wash Buffer IV/Wash Buffer (Centrifuge and remove old buffer after each wash). Re-suspend the cells in 100 µL of PBS. Proceed to DNA staining. If no DNA staining is desired, proceed to Microscopic or FACS analysis.
3. DNA staining: Add 2 µL of 1000X Total DNA Stain/Total DNA Stain (1000X) to 1998 µL PBS to prepare 1X Total DNA Stain, mix well and add 100 µL per well. Incubate the cells for 20 minutes at room temperature or at 4°C protected from light. Centrifuge and remove the stain solution; wash the cells once with 100 µL of PBS, centrifuge and remove PBS.

   Δ Note: Cells are compatible with all methods of slide preparation including wet mount or prepared mounting media.

7.5 Fluorescence microscope analysis:

Analyze samples for red fluorescence generated by TUNEL positive cells and green by total DNA respectively.

7.6 FACS analysis:

Harvest the cells by preferred method and wash with 0.5 ml of ice-cold PBS. Transfer the cell suspension into flow cytometry vessels. Analyze samples in FL-2 channel for signal generated by TUNEL positive cells during click reaction.

Δ Note: Trypsin can be used to collect the adherent cells prior to FACS analysis.

8. Typical Data

Data provided **for demonstration purposes only**.

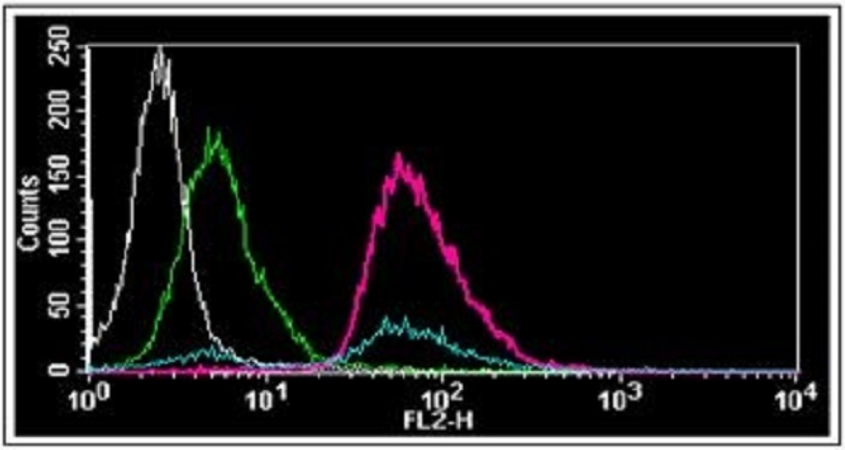

Figure 1. Detection of TUNEL-positive apoptotic strand breaks. Jurkat (Human T cell leukemia cell line from peripheral blood) cells-DNAse treated (10^6 cells/ml) induced strand breaks.

Unstained cells w/vehicle (white), background control cells processed for click reaction (green), negative control (untreated cells, TUNEL and click reaction; blue), DNase-treated cells (pink).

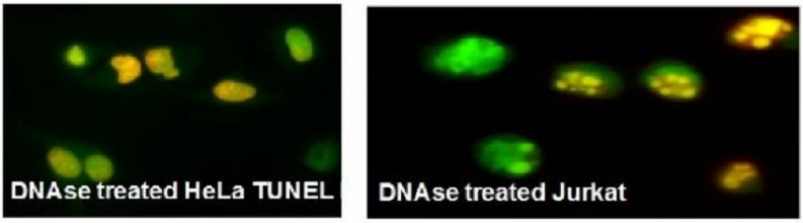

Figure 2.

Left panel: DNase treated HeLa (Human epithelial cell line from cervix adenocarcinoma) cells (10^5 cells/ ml). DNA staining and TUNEL and click reactions were performed.

Green: nuclear stain; Red (TUNEL positive); orange: apoptotic cells.

Right panel: DNase treated Jurkat (Human T cell leukemia cell line from peripheral blood) cells (10^6 cells/ ml). DNA staining and TUNEL and click reactions were performed.

Green: nuclear stain; Red (TUNEL positive); orange: apoptotic cells.

9. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

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