TransDetect® In Situ Click TUNEL Imaging Kit-488 Fluorophore

Please read the manual carefully before use.

Cat. No. FA301

Storage: at -20°C in the dark for one year

Description

TransDetect® In Situ Click TUNEL Imaging Kit-488 Fluorophore is a TUNEL assay reagent based on the Click reaction. In apoptosis, double-strand breaks or single strand breaks of genomic DNA can produce a large number of sticky 3'-OH ends. EdUTP is a dUTP nucleotide modified with a small alkyne moiety, which is more readily incorporated at the 3´-OH ends of fragmented DNA by the Terminal deoxynucleotidyl transferase (TdT) enzyme. A covalent reaction occurs between the azide and the alkyne moiety on EdUTP based on the Click reaction, and more sensitive TUNEL detection can be performed via fluorophore on the azide.

Features

- Extremely low cytotoxicity. No buffer system for highly toxic organoarsenic compounds required by common TUNEL reaction.
- High sensitivity and high specificity.
- Easy and fast operation.
- Suitable for the detection of different cell or tissue samples.

Kit Contents

Component	FA301-01 (10 rxns)	FA301-02 (50 rxns)
TdT Reaction Buffer-I (TRB-I)	5 ml	25 ml
EdUTP-I	100 μl	500 μl
TdT-I	150 μl	750 μl
Click Reaction Buffer 488 (CRB-488)	5 ml	25 ml
Reaction Buffer Additive (RBA)	250 μl	1.25 ml
Hoechst 33342	50 μl	250 μl
DNase I (3 units/μl)	50 μl	250 μl
10×DNase I Buffer	500 μl	2.5 ml

Protocol

Reagents not included in the kit

Product Name	Catalog
PBS (1×)	TransGen, Cat. FG701-01

- Formaldehyde fixing solution (4% paraformaldehyde in PBS)
- Permeabilisation solution (0.1% Triton X-100 in PBS)
- 3% BSA (prepared with 1× PBS)

Cell slides

Take 24-well cell culture plate as an example

1. Seed 1~2×10^5 cells into 24-well cell culture plate, culture or drug treatment overnight.
2. Take out the cell culture plate, discard the medium, and wash 2 times with 1 ml PBS.
3. Add 1 ml of formaldehyde fixing solution to each well, incubate for 15 minutes at room temperature, discard the fixing solution, and wash 2 times with 1 ml PBS.

4. Add 1 ml of permeabilisation solution to each well, incubate for 15 minutes at room temperature, discard the permeabilization solution, and wash 2 times with 1 ml PBS.
5. DNase I treatment (positive control, optional): Dilute 10×DNase I Reaction Buffer to 1×DNase I Reaction Buffer with permeabilisation solution, add DNase I (final concentration at 30 units/ml) and mix well. Place the prepared solution on ice and use it within 15 minutes. Add 500 μl per well, incubate for 30 minutes at room temperature, wash 2 times with PBS, 4 minutes each time.

Reaction Components	Volume
Permeabilisation Solution	445 μl
10×DNase I Reaction Buffer	50 μl
DNase I	5 μl
Total Volume	500 μl

6. TdT reaction: prepare the TdT reaction system according to the following table, place the prepared solution on ice and use it within 15 minutes. Add 500 μl of TdT reaction solution to each well and incubate for 1 h in a 37°C incubator, protected from light.

Reaction Components	Volume
TRB-I	475 μl
EdUTP-I	10 μl
TdT-I	15 μl
Total Volume	500 μl

7. Wash 2 times with 3% BSA, 4 minutes each time.
8. Prepare the staining solution according to the following table, place the prepared solution on ice and use it within 15 minutes. Add 500 μl of staining solution per well and incubate for 30 minutes at room temperature in the dark. Wash 3 times with PBS.

Reaction Components	Volume
CRB-488	475 μl
RBA	25 μl
Total Volume	500 μl

9. Prepare the Hoechst staining solution by diluting the Hoechst stock solution 1:1,000 in PBS. Add 500 μl of Hoechst staining solution to each well and incubate for 15 minutes at room temperature in the dark. Wash 3 times with PBS.

Tissue section (taking paraffin tissue cross section at a thickness of 4 μm from mouse spleen as an example)

1. Dewaxing and rehydration:
   After the section undergoes xylene dewaxing and gradient ethanol (95%, 90%, 85%, 75%, 50%) rehydration, immerse the section in PBS for 5 minutes, take out the section, aspirate the surrounding liquid and keep the surface of the section moist.
2. Add cell permeabilization solution, incubate at room temperature for 15 minutes, aspirate the permeabilization solution, and wash the section with PBS.
3. Same as steps 5-9 in cell slides.

Image and analyze

Assay fluorescence using fluorescence or confocal microscopy, and the 488 Fluorophore has an excitation maximum at 494 nm and an emission maximum at 520 nm.

Notes

• All components should be stored at -20°C, and reasonably arrange the experiment to avoid repeated freezing and thawing of each component.
• CRB-488 should be kept and used in the dark.

For research use only, not for clinical diagnosis.

Version number: V1-202209

Service telephone +86-10-57815020
Service email complaints@transgen.com