ABP Biosciences

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iClick™ TUNEL Andy Fluor™ 488 Imaging Assay
Catalog Number: A060

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
TdT reaction buffer (Component A)	**8 mL**	**1X solution**	• **-20 °C,** • **Protect from light**	**The product is stable for 1 year when stored as directed.**
TdT enzyme (Component B)	**100 µL**	**15 U/µL**		
EdUTP (Component C)	**50 µL**	**50X solution**		
iClick reaction buffer (Component D)	**6 mL**	**1X solution**		
iClick reaction buffer additive (Component E)	**50 mg**	**-**		
Hoechst 33342 (Component F)	**100 µL**	**1 mg/mL**		
DNase I (Component G)	**20 µL**	**2 U/µL**		
DNase I buffer (Component H)	**1.5 mL**	**1X solution**		

Number of assays: 50 assays.
Approximate fluorescence excitation/emission maxima: Andy Fluor 488: 495/520 nm.

Introduction

Internucleosomal cleavage of DNA is a hallmark of apoptosis. DNA cleavage in apoptotic cells can be detected in situ in fixed cells or tissue sections using the terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick-end labeling (TUNEL) method. TUNEL is highly selective for the detection of apoptotic cells but not necrotic cells or cells with DNA strand breaks resulting from irradiation or drug treatment.

In the TUNEL assay, TdT enzyme catalyzes the addition of modified dUTP to the 3'-OH ends of fragmented DNA. The modifications are fluorophores or haptens, including biotin or bromine which can be detected directly in the case of a fluorescently-modified nucleotide, or indirectly with streptavidin or antibodies, if biotin-dUTP or BrdUTP are used, respectively.

The new iClick™ TUNEL Andy Fluor™ 488 Imaging Assay utilize a dUTP modified with an alkyne, a small, bio-orthogonal functional group that enables the nucleotide to be more readily incorporated by TdT than other modified nucleotides such as biotin-dUTP and fluorescein-dUTP. Detection is based on a click reaction, a copper (I) catalyzed reaction between an azide and alkyne (Figure 1). The small size of the Andy Fluor™ azide (MW ~1,000) compared to that of an antibody (MW ~150,000) enables effortless penetration of complex samples with only mild fixation and permeabilization required. As a result, when compared to assays using other modified nucleotides, the iClick™ TUNEL Imaging Assay is fast (complete within 2 hours) and is able to detect a higher percentage of apoptotic cells under identical conditions.

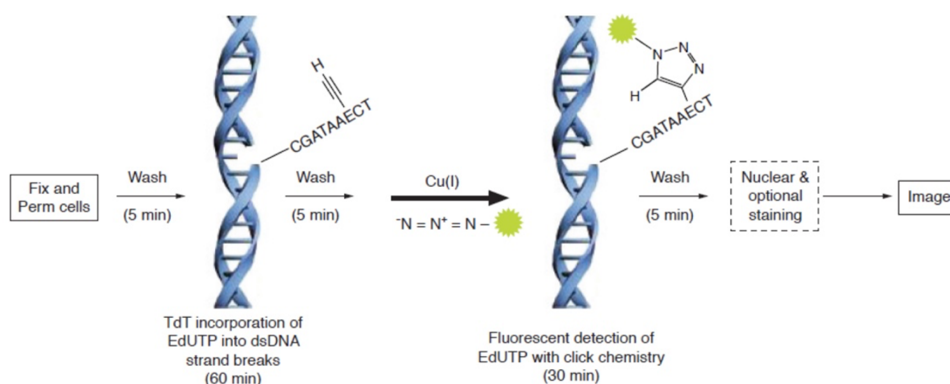

Figure 1. Detection of apoptosis with the iClick™ TUNEL Imaging Assay.

Materials Required but Not Provided

- Phosphate buffered saline (PBS)
- 4% formaldehyde in PBS (fixative)
- 0.2% Triton X-100 in PBS (permeabilization reagent)
- 3% BSA in PBS
- Deparaffinization solvents (optional)
- Proteinase K (optional)
- Antifade mounting medium

Experimental Protocols

Sample Preparation

1. Preparation of cultured cells or fresh-frozen tissue sections

Note: Apoptotic cells can detach from adherent cell cultures and be lost during wash steps. Culture supernatants may be stained using suspension cell protocols to detect detached apoptotic cells.

1.1 Wash cells or sections twice in PBS.

1.2 Fix cells or tissues in 4% formaldehyde in PBS (pH 7.4) for 30 minutes at 4ºC (not required for fixed-frozen sections).

1.3 Wash twice in PBS.

1.4 Permeabilize in PBS containing 0.2% Triton X-100 for 30 minutes at room temperature.

1.5 Wash twice in PBS.

2. Preparation of paraffin tissue sections

2.1 Deparaffinize and rehydrate tissue sections in Coplin jars at room temperature according to Tabel 2, below.

Table 2. Tissue deparaffinization procedure.

Xylenes	Xylenes	100% EtOH	100% EtOH	95% EtOH	85% EtOH	75% EtOH	1X PBS	1X PBS
5 min	5 min	5 min	5 min	5 min	3 min	3 min	5 min	5 min

2.2 Prepare 0.02 mg/mL of proteinase K in PBS.

2.3 Permeabilize sections with 100 µL of proteinase K solution for 30 minutes at room temperature. Proteinase K incubation time and temperature may require optimization depending on tissue type. Alternatively, microwave antigen retrieval protocols may be used at this step.

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2.4 Rinse in PBS. Wash 2 x 5 minutes in PBS.

Positive Control Preparation

Note: The DNase I generates strand breaks in the DNA to provide a positive TUNEL reaction.

3.1 Wash sample with deionized water.

3.2 Incubate samples with 50 µL DNase I buffer (Component H) for 10 minutes.

3.3 Prepare DNase I solution according to Table 3 and mix well.

Note: Do not vortex the DNase I solution as DNase I denatures with vigorous mixing.

Table 3. DNase I solution.

Reaction Components	Number of coverslips		
	1	2	3
DNase I (Component G)	1 µL	2 µL	3 µL
DNase I buffer (Component H)	49 µL	98 µL	147 µL
Total Volume	50 µL	100 µL	150 µL

3.4 Remove DNase I buffer and add 50 µL of the DNase I solution to each sample and incubate for 30 minutes at room temperature.

3.5 Wash sample once with deionized water.

TUNEL Reaction

4.1 Incubate samples with 100 µL TdT reaction buffer (Component A) for 10 minutes.

4.2 Immediately before use, prepare the TdT reaction cocktail according to Table 4.

Table 4. TdT reaction cocktails.

Reaction Components	Number of samples				
	1	2	4	8	10
TdT reaction buffer (Component A)	47 µL	94 µL	188 µL	376 µL	470 µL
TdT enzyme (Component B)	2 µL	4 µL	8 µL	16 µL	20 µL
EdUTP (Component C)	1 µL	2 µL	4 µL	8 µL	10 µL
Total Volume	50 µL	100 µL	200 µL	400 µL	500 µL

4.3 Remove TdT reaction buffer and add 50 µL TdT reaction cocktail to each sample, and allow the solution to spread completely over the surface.

a) For adherent cells or tissue sections, cover sample with a Parafilm coverslip to spread buffer evenly over cells or tissue section.

b) For negative control samples, add 50 µL TdT reaction cocktail without TdT Enzyme.

4.4 For cell staining, incubate for 60 minutes at 37ºC, protected from light. Tissue staining may require 2 hours incubation at 37ºC.

a) For adherent cells or tissue sections, perform incubation in a humid chamber.

b) For cells in suspension, perform incubation in a microplate on a rocking platform, or resuspend cells in reaction buffer every 15 minutes by gently flicking tubes.

4.5 Wash samples with 3%BSA in PBS for 2 x 5 minutes.

Click Detection

5.1 Prepare the iClick reaction buffer additive (Component E) by adding 625 µL of deionized water to the Component E vial. After use, aliquot and store any remaining solution at ≤-20°C. When stored as directed, the stock solution is stable for up to 1 year.

5.2 Prepare the iClick reaction cocktail according to Table 5 and mix well by vortexing.

Table 5. iClick reaction cocktails.

Reaction Components	**Number of samples**				
	1	**2**	**4**	**5**	**10**
iClick reaction buffer (Component D)	97.5 µL	195 µL	390 µL	487.5 µL	975 µL
iClick reaction buffer additive (prepared in step 5.1)	2.5 µL	5 µL	10 µL	12.5 µL	25 µL
Total Volume	100 µL	200 µL	400 µL	500 µL	1000 µL

5.3 Immediately add 100 µL of the iClick reaction cocktail (prepared in step 5.2) to each coverslip, and allow the solution to spread completely over the surface.

5.4 Incubate for 30 minutes at room temperature, protected from light.

5.5 Remove the iClick reaction cocktail and wash each coverslip with 3% BSA in PBS for 2 x 5 minutes.

5.6 (Optional) Stain with antibodies.

5.7 (Optional) Counterstain with Hoechst 33342. Dilute Hoechst 33342 (Component F) 1:500 in PBS to obtain a 1x Hoechst 33342 solution. Add 100 µL 1x Hoechst 33342 solution per coverslip or well and incubate for 15 minutes at room temperature, protected from light. Remove the Hoechst 33342 solution.

5.8 Wash each coverslip or well twice with PBS. Remove the wash solution.

5.9 Mount samples in antifade mouting medium for microscopy.

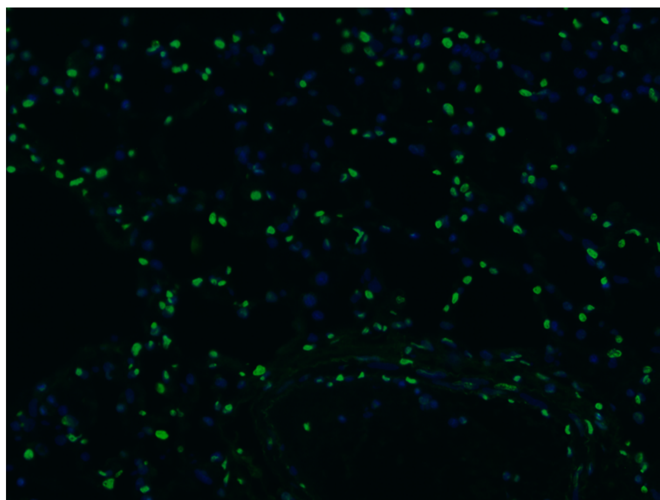

Figure 2. Detection of apoptotic cells in mouse tissue using iClick TUNEL Andy Fluor™ 488 Imaging Assay.