Revised: October 27, 2016

Product Information

CF™ Dye TUNEL Assay Apoptosis Detection Kits

Unit Size: 50 reactions

Kit Contents

Component	30063 CF™488A TUNEL Assay	30064 CF™594 TUNEL Assay	30074 CF™640R TUNEL Assay
TUNEL Equilibration Buffer	99965 5 mL	99965 5 mL	99965 5 mL
TUNEL Reaction Buffer	30063A 5 x 0.5 mL	30064A 5 x 0.5 mL	30074A 5 x 0.5 mL
TdT Enzyme	99964 50 uL	99964 50 uL	99964 50 uL

Spectral Properties

CF488A: Ex/Em 490/515 nm, spectrally similar to FITC or Alexa Fluor®488
CF594: Ex/Em 593/614 nm, spectrally similar to Texas Red®
CF640R: Ex/Em 642/662 nm, spectrally similar to Cy®5 or Alexa Fluor® 647
See Figure 1

Storage and Handling

Store TUNEL Assay Kit at -20ºC. Protect TUNEL Reaction Buffer from light. Avoid subjecting TUNEL Reaction Buffer to multiple freeze/thaw cycles. TdT Enzyme is a 50% glycerol stock and will not freeze at -20ºC; keep TdT Enzyme on ice during use. When stored as directed, the kit should be stable for at least 6 months from the date it is received.

CAUTION: TUNEL Equilibration Buffer and TUNEL Reaction Buffer contain cacodylate and cobalt chloride, which are toxic and carcinogenic by inhalation, ingestion, and skin contact. When using do not eat, drink, or smoke. After contact with skin, wash immediately with plenty of water. In case of exposure or if you feel unwell, seek medical advice immediately and show label if possible. Discard as regulated for toxic waste.

Product Description

Internucleosomal cleavage of DNA is a hallmark of apoptosis (1). Agarose gel electrophoresis of DNA from apoptotic cells reveals a characteristic ladder of 200 bp multimers. DNA cleavage in apoptotic cells also 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 (2).

In the TUNEL assay, TdT enzyme catalyzes the addition of labeled dUTP to the 3' ends of cleaved DNA fragments. Hapten-tagged dUTP (e.g. digoxigenin-dUTP or biotin-dUTP) can be detected using secondary reagents (e.g. anti-digoxigenin antibodies or streptavidin) for fluorescence or colorimetric detection. Alternatively, fluorescent dye-conjugated dUTP can be used for direct detection of fragmented DNA by fluorescence microscopy or flow cytometry. CF dye TUNEL assay kits contain dUTP conjugated to Biotium's exceptionally bright and photostable CF dyes, for bright fluorescent TUNEL staining using a rapid, direct labeling protocol.

References

1. Parrish and Xue (2006). Chromosoma 115: 89-97.
2. Gold et al. (1994). Lab Invest. 71(2):219-25.

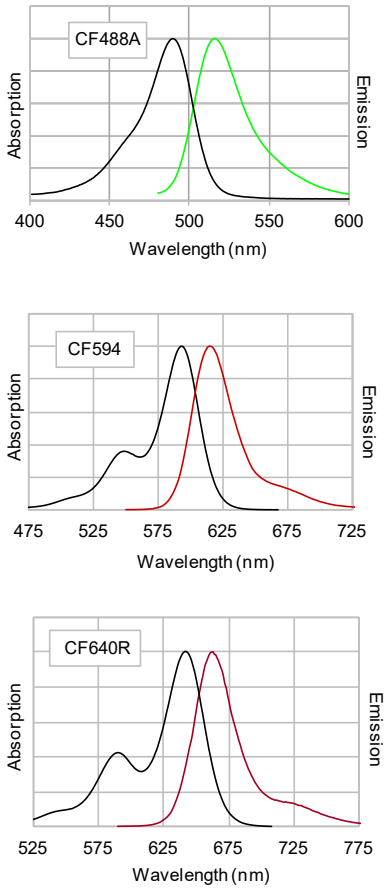

Figure 1. Normalized absorption and emission spectra for CF™488A, CF594, and CF640R conjugates.

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Labeling Protocol

1. Materials Required but not Provided

- Phosphate buffered saline pH 7.4 (PBS)
- 4% formaldehyde/PBS
- Triton™ X-100
- Bovine serum albumin (BSA) or normal goat or bovine serum
- 70% ethanol (optional)
- Deparaffinization solvents (paraffin sections only)
- Proteinase K (paraffin sections only)

2. Sample preparation

2.1 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.

a) Optional: include an extra sample to perform a negative control TUNEL reaction without TdT enzyme.
b) Wash cells or sections twice in PBS.
c) Fix cells or tissues in 4% formaldehyde in PBS (pH 7.4) for 30 minutes at 4ºC (not required for fixed-frozen sections)
e) Optional: store cells in 70% ethanol at -20ºC for up to two weeks, proceed to (f).
d) Wash twice in PBS.
e) Permeabilize in PBS containing 0.2% Triton X-100 for 30 minutes at room temperature.
f) Wash twice in PBS.

2.2 Preparation of paraffin tissue sections

a) Optional: include an extra sample to perform negative control (no TdT enzyme) TUNEL labeling.
b) Deparaffinize and rehydrate sections according to standard protocols.
c) Wash twice in PBS.
d) Permeabilize sections with 20 μg/mL proteinase K in PBS for 30 minutes at room 37ºC. Proteinase K incubation time and temperature may require optimization depending on tissue type. Alternatively, microwave antigen retrieval protocols may be used at this step.
e) Rinse in PBS. Wash 2 x 5 minutes in PBS.

3. TUNEL reaction

3.1 Incubate samples with 100 uL TUNEL Equilibration Buffer (Component 99965) for 5 minutes.

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

3.2 Immediately before use, prepare TUNEL reaction mix by adding 1 uL of TdT Enzyme (Component 99964) to 50 μL of TUNEL Reaction Buffer (Component A) for each labeling reaction.

3.3 Remove Equilibration Buffer and add 50 μL of TUNEL reaction mix to each sample.

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 without TdT enzyme, add TUNEL reaction buffer without TdT Enzyme.

3.4 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.

3.5 Wash samples 3 x 5 minutes in PBS containing 0.1% Triton X-100 and 5 mg/mL BSA (alternatively, 2% normal serum can be used in place of BSA).

3.6 Counterstain samples if desired. Mount samples in antifade mouting medium for microscopy, or analyze cells in suspension flow cytometry.

Expected results:

The nuclei of apoptotic cells should show bright fluorescence. No staining should be observed in samples treated without TdT enzyme.

Related Products

Catalog number	Product
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses
23007	TrueBlack Lipofuscin Autofluorescence Quencher, 20X in DMF
10405	NucView™405 Caspase-3 Substrate, 1 mM in DMSO
10402	NucView™ 488 Caspase-3 Substrate, 1 mM in DMSO
10403	NucView™ 488 Caspase-3 Substrate, 1 mM in PBS
30067	Dual Apoptosis Assay Kit with NucView™ 488 Caspase-3 Substrate & CF™594 Annexin V
30076	Dual Apoptosis Assay Kit with NucView™ 488 Caspase-3 Substrate & CF™640R Annexin V
30062	NucView™ 488 and MitoView™ 633 Apoptosis Kit
30072	NucView™ 488 and RedDot™2 Apoptosis and Necrosis Kit
30065	Apoptosis & Necrosis Quantitation Kit Plus
30066	Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus
30060	CF™488A Annexin V and 7-AAD Apoptosis Kit
30061	CF™488A Annexin V and PI Apoptosis Kit
30001	JC-1 Mitochondrial Membrane Detection Kit
80027	PathoGreen™ Histofluorescent Stain

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