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Product Information

CF® Dye TUNEL Assay Apoptosis Detection Kits

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

Unit Size: 50 reactions

Storage and Handling

Store TUNEL Assay Kit at -20°C. Protect TUNEL Reaction Buffer from light. Avoid subjecting TUNEL Reaction Buffer to multiple freeze/thaw cycles. TdT Enzyme is in a buffer containing 50% glycerol and will not freeze at -20°C; keep TdT Enzyme on ice during use. Kit components are stable for at least 6 months when stored as recommended.

CAUTION: TUNEL Equilibration Buffer and TUNEL Reaction Buffer contain cacodylate and cobalt chloride, which are toxic and carcinogenic by inhalation, ingestion, and skin contact. Handle using universal laboratory safety precautions and dispose as hazardous waste.

Product Description

Internucleosomal cleavage of DNA is a hallmark of apoptosis.[1] For example, agarose gel electrophoresis of DNA from apoptotic cells reveals a characteristic ladder of 200 bp multimers. DNA cleavage in apoptotic cells can also 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]

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. Fluorescent CF® Dye-conjugated dUTP can be used for direct detection of fragmented DNA by fluorescence microscopy or flow cytometry.

References

1. Chromosoma 115, 89-97 (2006); 2. Lab Invest. 71(2), 219-25 (1994).

Table 1. Technical Data

Dye	Ex/Em	Spectrally Similar Dyes
CF®488A	490/516	FITC
CF®594	593/615	Texas Red®
CF®640R	642/663	Cy®5

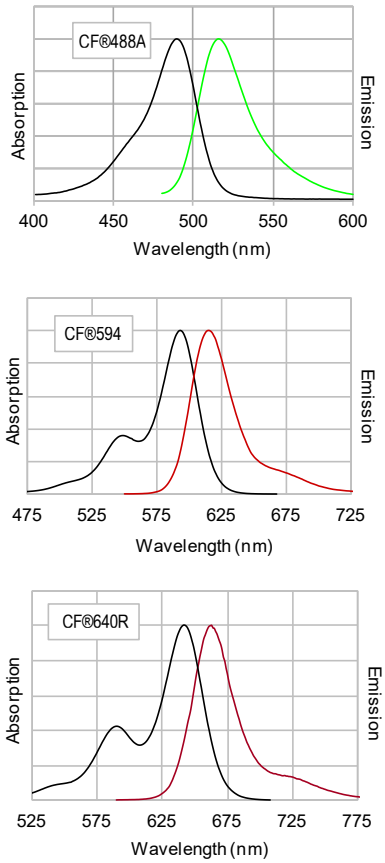

Figure 1. Normalized absorption and emission spectra for CF®488A, CF®594, and CF®640R conjugates.

Experimental Protocols

Considerations for staining

- TUNEL staining may be combined with Tyramide Signal Amplification (TSA), as the TSA amplification buffer should not impact the signal from CF® Dyes. However, we recommend performing TSA first to avoid the peroxide in the TSA buffer affecting the TUNEL signal.
- Immunofluorescence (IF) can be paired with TUNEL assays to detect specific proteins or markers associated with apoptosis, such as cleaved caspases or PARP. Together, these two methods provide a more comprehensive view of the apoptotic process. We typically recommend TUNEL assays be performed prior to IF staining.

Materials required but not provided

- Phosphate buffered saline (PBS) pH 7.4
- 4% Paraformaldehyde in PBS (Cat. No. 22023)
- Triton® X-100
- Bovine serum albumin (BSA, Cat. No. 22014), normal goat, or bovine serum
- 70% ethanol (optional)
- Deparaffinization solvents (paraffin sections only)
- Proteinase K (paraffin sections only)

Sample preparation

Option A: 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.

A1. Optional: Include an extra sample to perform a negative control TUNEL reaction without TdT enzyme.

A2. Optional: Positive control sections can be prepared by incubating fixed tissue sections with 10 U/mL DNase I (amplification grade) in 30 mM Tris pH 7.4, 4 mM $MgCl_2$, 0.1 mM DTT for 10 minutes at room temperature. Rinse several times in PBS and proceed with TUNEL staining. Include a control with no DNase I treatment for comparison.

A3. Wash cells or sections twice in PBS.

A4. Fix cells or tissues in 4% formaldehyde in PBS (pH 7.4) for 30 minutes at 4°C (not required for fixed-frozen sections).

A5. Optional: At this stage the cells or tissues can be stored in 70% ethanol at -20°C for up to 2 weeks.

A6. Wash twice in PBS.

A7. Permeabilize in PBS containing 0.2% Triton® X-100 for 30 minutes at room temperature.

A8. Wash twice in PBS.

Option B: Preparation of FFPE tissue sections

B1. Optional: Include an extra sample to perform negative control (no TdT enzyme) TUNEL labeling.

B2. Deparaffinize and rehydrate sections according to standard protocols.

B3. Wash twice in PBS.

B4. Permeabilize sections with 20 ug/mL proteinase K in PBS for 30 minutes at room temperature. Proteinase K incubation time and temperature may require optimization depending on tissue type. If incubation at room temperature is insufficient, it also can be done at 37°C.

Note: Other antigen retrieval protocols may also be performed at this step instead of proteinase K treatment. However, we recommend testing a positive control slide with both methods to confirm.

B5. Rinse in PBS. Wash 2 x 5 minutes in PBS.

TUNEL Reaction Protocol

1. Incubate samples with 100 uL TUNEL Equilibration Buffer (Cat. No. 99965) for 5 minutes.
2. Immediately before use, prepare TUNEL reaction mix by adding 1 uL of TdT Enzyme (Cat. No. 99964) to 50 uL of TUNEL Reaction Buffer for each labeling reaction.
3 . Carefully remove all traces of Equilibration Buffer and add 50 uL of TUNEL reaction mix to each sample.

   Note: For negative control samples, add TUNEL reaction buffer without TdT Enzyme.

4 . For adherent cells on coverslips or tissue sections, cover the sample with a Parafilm® coverslip to spread the reaction buffer evenly over the sample and prevent evaporation during incubation at 37°C.

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

   Notes:

   a. The reaction must be performed at 37°C. Labeling will not occur efficiently at room temperature.

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

   c. 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. We recommend 10^6 cells per sample in a 50 uL TUNEL reaction volume.

6. Wash samples 3 x 5 minutes in PBS.
7. Counterstain samples or perform IF labeling if desired. Mount samples in antifade mounting medium for microscopy, or analyze cells in suspension using flow cytometry.

Expected results

The nuclei of apoptotic cells should show bright fluorescence. There should not be staining in samples that were not treated with TdT enzyme.

Frequently Asked Questions (FAQs)

Question	Answer
I'm combining the CF® Dye TUNEL assay with another staining assay and prefer a hydrophobic pen, like the Super^{HT} PAP Pen 2.0, to Parafilm®. Is this compatible with this TUNEL assay?	Hydrophobic pens are compatible with this TUNEL assay. However, due to the small reaction volume, the tissue sections are likely to dry out during the 60 minute incubation at 37°C. Therefore, we recommend covering the sections with Parafilm® at least for the TUNEL reaction. You may perform your other staining steps as you usually would with a hydrophobic pen.
What is the source of TdT enzyme in the TUNEL Assay Kits?	Biotium's TUNEL Assay Kits contain contain TdT (calf thymus), a recombinant protein produced in *E. coli*.

Related Products

Cat. No.	Product
22023	4% Paraformaldehyde in PBS
22014	Bovine Serum Albumin 30% Solution
29001... 29085	Annexin V Conjugates
29088-29089	Recombinant Annexin V (Lyophilized)
29004R-5ug... 29085R-5ug	Annexin V CF® Dye Conjugates, Azide-Free, Lyophilized
30060	CF®488A Annexin V and 7-AAD Apoptosis Kit
30061	CF®488A Annexin V and PI Apoptosis Kit
10403... 10408	NucView® Caspase-3 Enzyme Substrates
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
70014	JC-1 (iodide salt)
70076	Aquaphile™ JC-1
30001	JC-1 Mitochondrial Membrane Detection Kit
70017	TMRM
40002... 40100	dUTP CF® Dye Conjugates
23008-23009	Drop-n-Stain EverBrite™ Mounting Medium (with or without DAPI)
23001-23002	EverBrite™ Mounting Medium (with or without DAPI)
23003-23004	EverBrite™ Hardset Mounting Medium (with or without DAPI)
23017-23018	EverBrite™ TrueBlack® Hardset Mounting Medium (with or without DAPI)
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO

Please visit our website at www.biotium.com to view our full selection of products for cell viability and apoptosis detection, along with hundreds of other products for cell biology, genomics, and proteomics research.