

Store at
-20°C

TUNEL Assay Kit (Fluorescence, 594 nm)

Cell Signaling
TECHNOLOGY®

#48513

1 Kit

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/supportOrders: 877-616-2355 (U.S.)
orders@cellsignal.com**For Research Use Only. Not For Use In Diagnostic Procedures.**

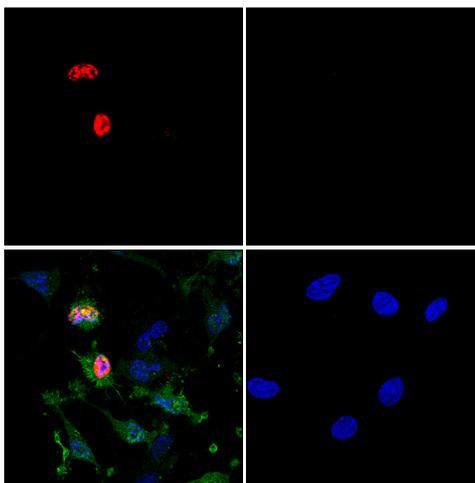
Product Includes	Product #	Kit Quantity	Storage Temp
TUNEL Assay Equilibration Buffer	84862	1 x 5 mL	-20°C
CF® 594 TUNEL Reaction Buffer	52143	5 x 500 µL	-20°C
TdT Enzyme	79533	1 x 50 µL	-20°C

Description: TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) is a method by which a fluorophore conjugated nucleotide is enzymatically linked to the 3' end of fragmented DNA. Since DNA fragmentation is a hallmark of apoptosis, the TUNEL assay has become a well established method to monitor apoptosis in situ.

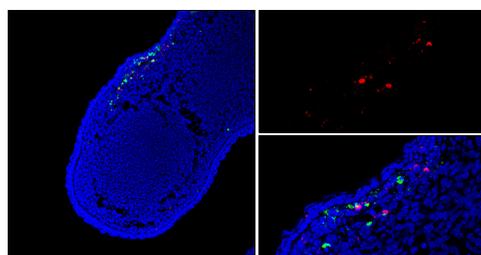
The TUNEL Assay Kit (Fluorescence, 594 nm) provides you with the buffers, enzyme, and dUTP fluorophore conjugate necessary to complete the TUNEL reaction in fixed cells or tissue. This kit is intended for use with fluorescence microscopy and/or flow cytometry.

Specificity/Sensitivity: Tissue processing or cell degradation may result in labeling of nuclei in non-apoptotic cells.

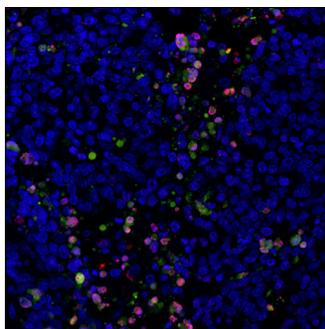
Background: Apoptosis is a regulated cellular suicide mechanism characterized by nuclear condensation, cell shrinkage, membrane blebbing, and DNA fragmentation (1). During late stages of apoptosis, DNA is fragmented by an endonuclease that cleaves the chromatin into nucleosomal units which can be visualized on gels as DNA laddering (2). DNA fragmentation also serves as a basis for monitoring apoptosis in situ using the TUNEL assay (3). In this assay, terminal deoxynucleotidyl transferase enzymatically incorporates a fluorophore conjugated nucleotide to the 3' end of fragmented DNA. Apoptosis can be monitored by an increase in TUNEL staining within intact cells.



Confocal analysis of HeLa cells, treated with Staurosporine #9953 (1 µM, 3 hr; left, positive) or untreated (right, negative), using TUNEL Assay Kit (Fluorescence, 594 nm) (red) followed by staining with Cleaved Caspase-3 (Asp175) Antibody #9661 (green) and DAPI #4083 (blue).



Confocal analysis of a fixed frozen E14.5 mouse digit at low magnification (left) and high magnification (right) using TUNEL Assay Kit (Fluorescence, 594 nm) (red) followed by staining with Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb #9664 (green) and DAPI #4083 (blue).



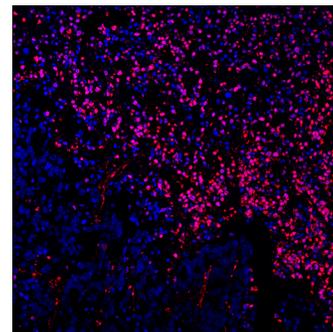
Confocal analysis of paraffin-embedded human colon carcinoma using TUNEL Assay Kit (Fluorescence, 594 nm) (red) followed by staining with Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb #9664 (green) and DAPI #4083 (blue).

Storage: All components in this kit are stable for at least 12 months when stored at the recommended temperature.

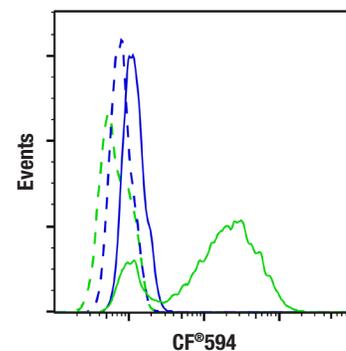
Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

- (1) Kerr, J.F. et al. (1972) *Br J Cancer* 26, 239-57.
- (2) Bortner, C.D. et al. (1995) *Trends Cell Biol* 5, 21-6.
- (3) Gavrieli, Y. et al. (1992) *J Cell Biol* 119, 493-501.



Confocal analysis of paraffin-embedded HT-29 xenograft using TUNEL Assay Kit (Fluorescence, 594 nm) (red) and DAPI #4083 (blue).



Flow cytometric analysis of Jurkat cells, untreated (blue) or treated with Staurosporine #9953 (1 µM, 3 hr; green), unlabeled (dashed lines) or labeled with TUNEL Assay Kit (Fluorescence, 594 nm) (solid lines).

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Fluorescent TUNEL Protocol for Cultured Cells and Frozen Tissues

A. Solutions and Reagents

Supplied Reagents

NOTE: Store at -20°C and avoid freeze/thaw cycles.

- TUNEL Equilibration Buffer** (1 bottle, 5 mL).
- CF[®] Dye TUNEL Reaction Buffer** (5 vials, 500 µL each): Protect from light.
- TdT Enzyme** (1 vial, 50 µL): Supplied as 50X concentrate. Keep on ice during use.

IMPORTANT: TUNEL Equilibration Buffer and CF[®] Dye TUNEL Reaction Buffer contain cacodylate and cobalt chloride. Handle in accordance with the SDS and discard as toxic waste.

Additional Reagents (Not Supplied)

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 10X Phosphate Buffered Saline (10X PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na₂HPO₄) and 2.4 g potassium phosphate, monobasic (KH₂PO₄) to 1 L dH₂O. Adjust pH to 7.4.
- 1X Phosphate Buffered Saline (PBS):** To prepare 1 L add 100 mL 10X PBS to 900 mL dH₂O.
- 1X Phosphate Buffered Saline with Triton X-100 and BSA (PBS-TB):** To prepare 100 mL 1X PBS-TB, add 10 mL 10X PBS, 200 µL Triton X-100, and 0.5 g BSA (#9998) to 90 mL dH₂O, mix. Adjust pH to 7.4.
- 4% Formaldehyde, Methanol-Free (#47746):** Use fresh.

B. TUNEL Procedure

General Considerations:

- Samples should be kept in a humidity chamber while running the TUNEL assay. Do not allow samples to dry at any time during this procedure.
- All subsequent incubations should be carried out at room temperature (20-25°C) unless noted otherwise.
- Apoptotic cells in adherent cultures are loosely adherent and may be lost during washing. These can be captured in the supernatant and used if desired.

Fixation and Permeabilization

- 1. Fixation:** Samples should be fixed using a gentle cross-linking fixative.
 - a. **Cells:** incubate in 4% formaldehyde for 15 - 30 minutes.
 - b. **Unfixed frozen tissue:** after cryosectioning, briefly allow samples to come to room temperature and dry, then incubate in 4% formaldehyde for 15 - 30 minutes.
 - c. **Fixed frozen tissue:** proceed directly to permeabilization below (step 3).
- 2. Wash:** Rinse two times in PBS for 5 min each.
- 3. Permeabilize:** Incubate in PBS-TB for 30 min.
- 4. Wash:** Rinse two times in PBS for 5 min each.

TUNEL Reaction

- 1. Preparation:** Incubate samples with 100 µL (or enough to cover the sample) TUNEL Equilibration Buffer for 5 min.
- 2. Detection:** Immediately before use, prepare TUNEL reaction mix by adding 1 µL of TdT Enzyme to 50 µL of TUNEL Reaction Buffer for each labeling reaction. Remove Equilibration Buffer and add 50 µL (or enough to cover the sample) of TUNEL reaction mix to each sample.
- 3. Incubate:** For cell staining, incubate for 60 min at 37°C, protected from light. Tissue staining may require up to 2 hours of incubation at 37°C also protected from light.
- 4. Wash:** Rinse three times in PBS-TB for 5 min each.
- 5. Co-label:** If desired, proceed with immunostaining. Consult with the manufacturer's recommendations to confirm the primary antibody is compatible with this protocol's fixation and permeabilization conditions. Otherwise continue on to step 6.
- 6. Mount:** Mount samples in a compatible anti-fade medium such as ProLong[®] Gold Antifade Reagent (#9071).

Fluorescent TUNEL Protocol for Paraffin Embedded Samples

A. Solutions and Reagents

Supplied Reagents

NOTE: Store at -20°C and avoid freeze/thaw cycles.

- 1. TUNEL Equilibration Buffer** (1 bottle, 5 mL)
- 2. CF[®] Dye TUNEL Reaction Buffer** (5 vials, 500 µL each): Protect from light.
- 3. TdT Enzyme** (1 vial, 50 µL): Supplied as 50X concentrate. Keep on ice during use.

IMPORTANT: TUNEL Equilibration Buffer and CF[®] Dye TUNEL Reaction Buffer contain cacodylate and cobalt chloride. Handle in accordance with the SDS and discard as toxic waste.

Additional Reagents (Not Supplied)

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 1. Xylene**
- 2. Ethanol, anhydrous denatured, histological grade** (100% and 95%).
- 3. 10X Phosphate Buffered Saline (10X PBS)** : To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na₂HPO₄) and 2.4 g potassium phosphate, monobasic (KH₂PO₄) to 1 L dH₂O. Adjust pH to 7.4.
- 4. 1X Phosphate Buffered Saline (PBS)** : To prepare 1 L add 100 mL 10X PBS to 900 mL dH₂O.
- 5. 1X Phosphate Buffered Saline with Triton X-100 and BSA (PBS-TB)**: To prepare 100 mL 1X PBS-TB, add 10 mL 10X PBS, 200 µL Triton X-100, and 0.5 g BSA (#9998) to 90 mL dH₂O, mix. Adjust pH to 7.4.
- 6. Proteinase K Solution:** For use dilute Proteinase K (#10012) to 20 µg/ml in dH₂O.
- 7. 1X Citrate Unmasking Solution (optional alternative to Proteinase K Solution):** To prepare 250 ml of 1X citrate unmasking solution, dilute 25 ml of SignalStain[®] Citrate Unmasking Solution (10X) (#14746) with 225 ml of dH₂O.

B. TUNEL Procedure

General Considerations:

- Samples should be kept in a humidity chamber while running the TUNEL assay. Do not allow samples to dry at any time during this procedure.
- All subsequent incubations should be carried out at room temperature (20-25°C) unless noted otherwise.
- Heat-mediated antigen retrieval is recommended if co-labeling with an antibody is attempted.

Sample Preparation

1. Deparaffinize/rehydrate:

- a. Incubate sections in three washes of xylene for 5 min each.
- b. Incubate sections in two washes of 100% ethanol for 10 min each.
- c. Incubate sections in two washes of 95% ethanol for 10 min each.

2. Wash:

 Rinse two times in PBS for 5 min each.

3. Antigen Retrieval:

 Incubate samples with Proteinase K solution for 30 min. Incubation time and temperature may require optimization depending on tissue type.

- a. Alternatively Citrate Unmasking Solution can be used: Heat slides in a microwave submersed in 1X citrate unmasking solution until boiling is initiated; follow with 10 min at sub-boiling temperature (95°-98°). Cool slides on bench top for 30 min.

4. Wash:

 Rinse two times in PBS for 5 min each.

TUNEL Reaction

- 1. Preparation:** Incubate samples with 100 µL TUNEL Equilibration Buffer or enough to cover the sample for 5 min.
- 2. Detection:** Immediately before use, prepare TUNEL reaction mix by adding 1 µL of TdT Enzyme to 50 µL of TUNEL Reaction Buffer for each labeling reaction. Remove Equilibration Buffer and add 50 µL (or enough to cover the sample) of TUNEL reaction mix to each sample.
- 3. Incubate:** Incubate for 60 min at 37°C, protected from light. Tissue sections may require 2 hours of incubation at 37°C also protected from light.
- 4. Wash:** Rinse three times in PBS-TB for 5 min each.
- 5. Co-label:** If desired, proceed with immunostaining for co-labeling. Consult with the manufacturer's protocol for the recommended staining conditions. Otherwise continue on to step 6.
- 6. Mount:** Mount samples in a compatible anti-fade medium such ProLong[®] Gold Antifade Reagent (#9071).

Fluorescent TUNEL Protocol for Flow Cytometry

A. Solutions and Reagents

Supplied Reagents

NOTE: Store at -20°C and avoid freeze/thaw cycles.

- 1. TUNEL Equilibration Buffer** (1 bottle, 5 mL).
- 2. CF[®] Dye TUNEL Reaction Buffer** (5 vials, 500 µL each): Protect from light.
- 3. TdT Enzyme** (1 vial, 50 µL): Supplied as 50X concentrate. Keep on ice during use.

IMPORTANT: TUNEL Equilibration Buffer and CF[®] Dye TUNEL Reaction Buffer contain cacodylate and cobalt chloride. Handle in accordance with the SDS and discard as toxic waste.

Additional Reagents (Not Supplied)

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 1. 1X Phosphate Buffered Saline (PBS):** To prepare 1 L 1X PBS: add 100 ml 10X PBS (#12528) to 900 ml water.
- 2. 4% Formaldehyde, Methanol-Free** (#47746)
- 3. 100% Methanol (#13604), or Cell Permeabilization Buffer (Triton™ X-100)** (#39487)
- 4. (OPTIONAL) Antibody Dilution Buffer:** Purchase ready-to-use Flow Cytometry Antibody Dilution Buffer (#13616), or prepare a 0.5% BSA PBS buffer by dissolving 0.5 g Bovine Serum Albumin (BSA) (#9998) in 100 ml 1X PBS. Store at 4°C.

B. TUNEL Procedure

General Considerations:

- Do not allow samples to dry at any time during this procedure.
- All incubations should be carried out at room temperature (20-25°C) unless noted otherwise.

Fixation

NOTE: Adherent cells or tissue should be dissociated and in single-cell suspension prior to fixation.

NOTE: Optimal centrifugation conditions will vary depending upon cell type and reagent volume. Generally, 150-300g for 1-5 minutes will be sufficient to pellet the cells.

NOTE: If using whole blood, lyse red blood cells and wash by centrifugation prior to fixation.

1. Pellet cells by centrifugation and remove supernatant.
2. Resuspend cells in approximately 100 µl 4% formaldehyde per 1 million cells. Mix well to dissociate pellet and prevent cross-linking of individual cells.
3. Fix for 15 min at room temperature (20-25°C).
4. Wash by centrifugation with excess 1X PBS. Discard supernatant in appropriate waste container. Resuspend cells in 0.5-1 ml 1X PBS. Proceed to Permeabilization step.
 - a. Alternatively, cells may be stored overnight at 4°C in 1X PBS.

Permeabilization

NOTE: Permeabilization is required for the completion of the TUNEL reaction. If immunostaining will be performed as part of the TUNEL assay, select a permeabilization method that is compatible with the antibody or antibodies used in your assay.

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol.
2. Permeabilize for a minimum of 10 min on ice.
3. Proceed with TUNEL Reaction or store cells at -20°C in 90% methanol.

Alternatively, permeabilize with Cell Permeabilization Buffer (Triton™ X-100) (#39487), using approximately 100 µl per 1 million cells. Incubate 10 min at room temperature.

TUNEL Reaction

NOTE: Count cells using a hemocytometer or alternative method.

1. If necessary, aliquot desired number of cells into tubes or wells. (Generally, 5x10⁵ to 1x10⁶ cells per assay.)
2. Wash cells by centrifugation in excess 1X PBS. Discard supernatant in appropriate waste container.
3. Incubate samples with 100 µl TUNEL Equilibration Buffer for 5 min.
4. Immediately before use, prepare TUNEL reaction mix by adding 1 µl of TdT Enzyme to 50 µl of TUNEL Reaction Buffer for each labeling reaction. Pellet cells via centrifugation, remove Equilibration Buffer and add 50 µl of TUNEL reaction mix to each sample.
5. Incubate for 60 min at 37°C, protected from light.
6. Wash by centrifugation in 1X PBS. Discard supernatant. Repeat.
7. If desired, proceed with immunostaining. Otherwise proceed to step 8.
8. Resuspend cells in 200 – 500 µl of 1X PBS and analyze on flow cytometer.