1.2.3 Tips for avoiding or eliminating potential TUNEL labeling artifacts

To avoid this artifact	Which may be caused by	Try the following
Nonspecific TUNEL labeling	● DNA strand breaks induced by UV irradiation during tissue embedding (UV used to polymerize tissue embedding material such as methacrylate)	● Use a different embedding material, which does not require UV irradiation ● Use an alternate polymerization method
	● Acid tissue fixatives (*e.g.,* mathacarn, Carnoy's fixative) cause DNA strand breaks	● Use buffered 4% paraformaldehyde as fixative
	● Endogenous nuclease activity which occurs soon after tissue preparation (*e.g.,* in smooth muscle tissue slices)	● Fix tissue immediately after organ harvest ● Perfuse fixative through liver vein in intact animal
	● TdT concentration too high during TUNEL labeling	● Reduce concentration of TdT by diluting it 1:2 or 1:3 with TUNEL Dilution Buffer (Cat.No. 1966 006) containing 30 mM Tris (pH 7.2) containing 140 mM sodium cacodylate and 1 mM $CoCl_2$
	● Endogenous alkaline phosphatase activity during converter reaction	● Block endogenous AP activity by adding 1 mM levamisole to the AP substrate solution
	● Endogenous peroxidase activity during converter reaction	● Before permeabilizing cells, block endogenous POD activity by immersing the slides in a solution of 0.3% H_20_2 in methanol
	● Nonspecific binding of anti-fluorescein antibody conjugate during converter reaction	● Block nonspecific sites with normal anti-sheep serum ● Block nonspecific sites with PBS containing 3% BSA (20 min) ● Use 1:2 dilution of converter solution in PBS
High background	● Formalin fixation, which causes yellow staining of cells containing melanin precursors	● Use methanol fixation ● ***Note:*** *This fixation may lead to a reduction in TUNEL labeling sensitivity*
	● TUNEL labeling mix too concentrated (*e.g.,* for carcinomas)	● Reduce concentration of labeling mix by diluting it 1:2 with TUNEL Dilution Buffer (Cat. No. 1966 006) containing 30 mM Tris (pH 7.2) containing 140 mM sodium cacodylate and 1 mM $CoCl_2$
	● Endogenous alkaline phosphatase activity during converter reaction	● Block endogenous AP activity by adding 1 mM levamisole to the AP substrate solution
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To avoid this artifact	Which may be caused by	Try the following
Low TUNEL labeling (low sensitivity)	Ethanol and methanol fixation	Use buffered 4% paraformaldehyde as fixative
	Extensive crosslinking during prolonged fixation reactions	Reduce fixation time Use buffered 2% paraformaldehyde as fixative
	Insufficient permeabilization of cells, so TUNEL reagents cannot reach nuclei	Pretreat with proteinase K (concentration and time must be optimized empirically) ***Note:*** *To avoid possible nuclease contamination, use only Proteinase K from Roche Applied Science, Cat. No. 03 115 836 001* Pretreat with 0.01 M sodium citrate for 30 min at 70°C Increase TUNEL incubation time
	Restricted access of TUNEL reagents to nuclei, caused by paraffin-embedding	After dewaxing tissue sections, treat with proteinase K (concentration, time, and temperature must be optimized empirically) ***Note:*** *To avoid possible nuclease contamination, use only Proteinase K from Roche Applied Science, Cat. No 03 115 836 001* Immerse dewaxed tissue sections in 200 ml 0.01 M citrate buffer (pH 6.0) and treat with microwave irradiation (370 W, 5 min) ***Note:*** *Conditions must be experimentally optimized for each tissue*
No signal on positive control	Inadequate DNase treatment (DNase concentration too low)	For cryosections, apply 1 µg/ml DNase For paraffin-embedded tissue sections, apply 0.5 mg/ml DNase For many other samples, apply 1 U/ml DNase in a solution of 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 5 mM $MnCl_2$, 0.1 mM $CaCl_2$, 25 mM KCl; incubate 30 min at 37°C As an alternative DNase buffer, use a solution of 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mg/ml BSA
Diminished TUNEL staining during DNA counterstaining	Quenching of fluorescein signal by propidium iodide (PI)	Use 0.5 µg/ml PI as DNA stain Substitute TO-PRO-3 (from Molecular Probes) in place of PI