Materials List for:

Detection and Analysis of DNA Damage in Mouse Skeletal Muscle *In Situ* Using the TUNEL Method

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Materials

Name	Company	Catalog Number	Comments
4% Paraformaldehyde in phosphate buffered saline	Electron Microscopy Sciences	19202	For procedures described here, 4% solution was prepared fresh from powder. Paraformaldehyde from any supplier may be used. Prepared formaldehyde solution should be stored at 4 °C and should not be used after its expiration date (up to several months). Paraformaldehyde is a carcinogen and a toxin by inhalation and skin contact. Please follow precautions specified in the MSDS when handling paraformaldehyde.
Sucrose	Sigma	S0389	Used for cryoprotecting tissue before freezing. Sucrose from any supplier may be used.
O.C.T. compound	Tissue-Tek	4583	Embedding medium for cryosectioning.
Cryostat	Leica	CM 3050S	A Leica CM3050S cryostat was used for the preparations described here. Any cryostat capable of cutting 10 µm sections may be used.
Glass slides, 25 x 75 x 1 mm	Fisher	12-552-3	Slides from any supplier may be used.
Gelatin	Sigma	G-9391	Gelatin is used to promote tissue section adhesion to glass slides. To coat glass slides with gelatin, dissolve 2.75 g gelatin and 0.275 g chrome alum in 500 ml distilled water, warm to 60 °C, dip slides for several seconds, and let dry. Gelatin from any supplier may be used. Alternatively, gelatin-precoated slides may be purchased.
Chromium(III) potassium sulfate dodecahydrate (chrome alum)	Sigma	243361	Chrome alum is added to gelatin solution to promote tissue adhesion on glass slides. It is a possible carcinogen and a toxin by inhalation and skin contact. Please follow precautions specified in the MSDS when handling chrome alum.
Vectabond tissue adhesion reagent	Vector Labs	SP-1800	Optional substrate for better tissue adhesion to glass slides; gelatin-coated slides may be used instead.

Tween20	Sigma	P9416	A detergent used to permeabilize tissue. Tween20 from any supplier may be used.
Triton X100	Sigma	T8787	A detergent used to permeabilize tissue. Triton X100 from any supplier may be used.
TACS 2 TdT fluorescein *in situ* apoptosis detection kit	Trevigen	4812-30-K	Commercial kit for fluorescence-based TUNEL labeling.
DNase/nuclease	Trevigen	4812-30-K	(included with kit)
DNase/nuclease buffer	Trevigen	4812-30-K	(included with kit)
10x phosphate buffered saline (PBS), pH 7.4	Amresco	780	Make 1x PBS for washes and dilutions. PBS from any supplier may be used.
DNase-free water	Quality Biologicals	351-029-131	Water from any supplier may be used.
Hoechst 33258	Sigma	94403	Nuclear dye. Any blue fluorescent nuclear dye may be used. As a DNA-binding dye, Hoechst is a suspected carcinogen and should be handled with protective equipment to minimize skin contact.
Parafilm M	multiple	807	Any other hydrophobic film or cover slip may be used. Available from multiple suppliers.
Fluorescent microscope with digital camera	--	--	Any fluorescent microscope capable of digitally capturing red, green, and blue fluorescence in separate channels may be used.
Vectashield antifade media	Vector Labs	H-1000	Antifade media from any supplier may be used.
glass coverslips, No.1 thickness	Brain Research Labs	2222-1	Cover slips from any supplier may be used. The smallest size of 22 x 22 mm is sufficient for neonatal mouse leg sections.
Nail polish	Ted Pella	114-8	Used to seal coverslips. Nail polish from any supplier (including regular retailers) may be used. Avoid using nail polish with color or additives that may reflect light during fluorescent imaging.