

# An Assay to Detect Protection of the Retinal Vasculature from Diabetes-Related Death in Mice

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jove.com/video/66123

## Materials

Name	Company	Catalog Number	Comments
10% neutral buffered formalin	Fischer Scientific	SF100-4	Fixation
24-well plates	Falcon	353047	
33 G needle	Hamilton	customized	
Ammonium hydroxide	Sigma	221228-1L-PCA	
C57/BL6/J mice	The Jackson Laboratory	Jax #000664	
Cytokine cocktail			consisted of a 1:1:1 ratio of 1 µg/mL TNF-α, 1 µg/mL IL-1 β and and 1500 U/µL IFN- γ
Dissecting microscope			Any microscope that allows good visualization of the retina is adequate.
Dumont #3 forceps	Fine Science Tools (FST)	11231-30	Straight tips
Dumont #5 forceps	Fine Science Tools (FST)	11253-25	Micro-blunted tips
Easy-Grip Tissue Culture Dishes	Falcon	353001	35 x 10 mm
Glass transfer pipet	Fischer Scientific	1367820A	snap off the thin end of a Pasteur pipet and fit the "broken" end with a rubber bulb.
Harris modified hematoxylin	Sigma	HHS32	
Image J	NIH, Bethesda		<a href="https://imagej.nih.gov/ij/">https://imagej.nih.gov/ij/</a>
In Situ Cell Death Detection Kit, Fluorescein	Milipore	11684795910	TUNEL reaction mixture
Micro cover glasses	VWR	48366-227	22 mm x 22 mm
Microknife	Sharpnoint	72-1551	
Micro-spatula	Fine Science Tools	10091-12	
Mounting cassette			Any transparent cassette that is slightly bigger than the microscope slide
Periodic acid	Sigma	3951	
Periodic acid solution			35 mM periodic acid with 12 mM sodium acetate in H <sub>2</sub> O
Permount mounting medium	Fischer Scientific	SP15- 100	

Prism 9	GraphPad		
Prolog Gold antifade reagent with DAPI	Invitrogen	P36935	
Recombinant human IFN- $\gamma$	Peptotec	300-02	
Recombinant human IL-1 $\beta$	Peptotec	200-01B	
Recombinant human TNF- $\alpha$	Peptotec	300-01A	
Schiff reagent base	Sigma	3952016	
Shaker Incubator (belly button shaker)	IBI Scientific	BBUAAUV1S	
Sodium acetate	Sigma	71196	
Steritop sterile vacuum bottle	Millipore	SCGPS05RE	Create filtered water
Superfrost Plus treated microscope slides	Fischer Scientific	12-550-15	use slides from unopened box
Tert-butyl hydroperoxide (TBH)	Sigma	75-91-2	
TRIZMA base	Fischer Scientific	11-101-5522	make 100 mM Tris, adjust pH to 7.8 using HCl)
Trypsin 1:250	Amresco	0458-50G	
Two "brushes"			made from single black hair taped to the end of plastic transfer pipet. One brush with a free end. The other brush with a loop
Vannas Spring Scissors	Fine Science Tools (FST)	15000-00	
Xylene	Sigma	65351-M	
YL trypsin solution			3% trypsin in 0.1 M Tris (pH 7.8)
Zeiss LSM 710 fluorescence microscope	Zeiss Microscopy		