Materials List for:

Techniques for Processing Eyes Implanted With a Retinal Prosthesis for Localized Histopathological Analysis

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Materials

Name	Company	Catalog Number	Comments
The Davidson marking system	Bradley Products	1163-4 - red 1163-5 - blue 1163-2 - yellow 1163-1- green	
Rabbit Polyclonal Anti-Glial Fibrillary Acidic Protein Antibody	Millipore	AB5804	1:1500, overnight incubation
Mouse Monoclonal Anti-Glutamine Synthetase Antibody	Millipore	MAB302	1:100 overnight incubation
Monoclonal Mouse Anti-Neurofilament 200 Antibody	Sigma-Aldrich	N0142	1:100 overnight incubation
4',6-diamindino-2-phenylindole, dilactate (dilactate)	Life Technologies	D3571	1:25,000 30 min incubation
Alexa Fluor 488 goat anti-mouse IgG	Life Technologies	A11029	1:500
Superfrost 90° yellow	Grale Scientific	SF41299	
FLEX IHC Microscope Slides	DAKO	K802021-2	
Alexa Fluor 594 goat anti-rabbit IgG	Life Technologies	A11037	1:500
Normal goat serum	Life Technologies	PCN5000	10% serum/0.1% Triton X-100/PBS

Non-Standard Solution Preparation for Special Stains

1% Aqueous Cresyl Violet Stock Solution

- Cresyl violet 1 g
- Distilled water 100 ml

Cresyl Violet Acetate Working Solution

- 1% aqueous cresyl violet stock solution 9 ml
- Distilled water 51 ml
- 10% acetic acid 0.5 ml

Biebrich Scarlet-Acid Fuchsin Solution

- 1% Beihrich scarlet 90 ml
- 1% acid fuchsin 10 ml
- Glacial acetic acid 1 ml

Phosphomolybdic-Phosphtungstic Acid Solution

- Phosphomolybdic acid 2.5 g
- Phosphotungstic acid 2.5 g
- Distilled water 100 ml

Aniline Blue Solution

- Aniline blue 2.5 g
- Glacial acetic acid 2 g
- Distilled water 100 ml

Solutions for Immunohistochemistry

Wash Buffer Solution

- 0.1% Triton X-100 in Phosphate buffered saline (PBS)

Serum Block Solution

- 10% normal goat serum/0.1% Triton X-100 in PBS

SUPPLEMENTARY MATERIAL

Luxol Fast Blue

The purpose of performing a Luxol Fast Blue (LFB) stain was to identify the ganglion cells in the ganglion cell layer through the counterstain of cresyl violet. While LFB stains myelin, the cresyl violet stain binds to the Nissl substances in neurons, composed of rough endoplasmic reticulum and ribosomes. Numerous cells in the retina contain Nissl substance including amacrine cells. These cells usually are located in the inner boundary layer of the inner nuclear layer, but displaced amacrine cells can be found in the ganglion cell layer. The staining of Nissl substance is helpful in differentiating large, heavily stained ganglion cells from smaller displaced amacrine cells. To aid visualisation of these cells, we modified the cresyl violet working solution protocol by increasing the volume of the cresyl violet stock solution in the working solution. We increased the volume in 1.0 ml increments from 6.0 ml to 9.0 ml, in turn reducing the volume of distilled water. Assessing the ease of visualising and identifying the ganglion cells, optimal staining was achieved with 9 ml of additional cresyl violet stock solution and 51 ml of distilled water. Cresyl violet is a basic dye, though to dye the Nissl substance, it is required to be used in an acidic solution, and therefore the volume of acetic acid remained unchanged at 0.5 ml.

Periodic Acid Schiff

The Periodic Acid Schiff (PAS) stain was performed to highlight polysaccharides especially glycogen, found in basement membranes and fungal cell walls, indicating a fungal infection. The process of the PAS stain is to oxidise the hydroxyl groups in polysaccharides using periodic acid, producing aldehyde groups. The application of the Schiff reagent, binds to the aldehyde groups producing a magenta color with haematoxylin counterstaining producing purple cell nuclei. Our slides showed weak staining at the inner limiting membrane. This may be due to the polysaccharides present, as not all polysaccharides can be oxidised with a standard time frame, especially those containing anionic polysaccharides. We therefore increased the duration of the oxidation step from 10 min to 12, 15, and 20 min. We found that a 15 min oxidation step was most effective in PAS staining.

Masson's Trichrome Blue

The principle of the Masson's trichrome blue stain is to stain collagen and muscle, which in our retinal slides can be used to indicate an increase in collagen tissue which may indicate fibrosis due to injury. This stain is sensitive to fixation techniques, so alterations were needed to obtain optimal staining. The process of this stain is to initially stain cytoplasm and muscle fibres red using the acidic Biebrich scarlet-acid fuchsin red dye. Differentiation with phosphomolybdic/phosphotungstic acid competes with the red dye in the collagen fibres. In the sclera, the large phosphomolybdic/phosphotungstic acid molecules are able to penetrate the loose connective tissue, as opposed to the dense muscle fibres which are penetrable to small molecules only. Aniline blue dye is then applied to replace the acid in the collagen fibres producing blue dyed sclera. To optimise this stain in retinal sections, the duration of staining with Biebrich scarlet-acid fuchsin dye was reduced to create a balance between the blue and the red dye. The use of Davidson's fixative also required an extended differentiation time to remove the red dye from the collagen. We trialled differentiation at 5, 6, 8, and 10 min, with optimal results occurring at 6 min. Differentiation also varies on the thickness and cutting of smooth sections, with greater than 6 min differentiation possibly being required.

Glial Fibrillary Acidic Protein (GFAP) Antibody

Polyclonal, host species is rabbit, has a molecular weight of 50 kDa. GFAP antigen is an intermediate filament found in the cytoplasm (the cytoplasm of astrocytes and Müller cells in the retina), the antigen is made form 428 amino acids and has a molecular weight of 49512 Da.

Neurofilament-200 antibody

Monoclonal, host species is mouse, clone N52, isotype IgG1, it recognises phosphorylated and non-phosphorylated forms of the heavy neurofilaments which have a molecular weight of 200 kDa. The antibody will bind to an epitope in the tail domain of the neurofilament. The antibody will stain neurofilaments in the neuronal perikarya, dendrites and axons.

Glutamine Synthetase (GS) antibody

Monoclonal, clone GS-6, host species is mouse, has a molecular weight of 45 kDa and antibody isotype IgG2a. The GS antigen is found in the cell cytoplasm (cytoplasm of Müller cells in the retina), the antigen has 373 amino acids and a molecular weight of 42,064 Da.