## The University of Iowa Dezii Translational Vision Research Group

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SOP Number:	D-DEZ-PRO-010	Revision Number: 0	
		Effective Date: 07 Apr 2015	
Author:		Date:	
Daviewer:		Date:	
QA Approval:		Date:	

#### A. OBJECTIVE

This SOP is to provide information for the preparation of mounting media in the DTVR cGMP group.

#### B. APPLICABILITY

This procedure shall be performed by a trained scientist or technician in the DTVR laboratories.

#### C. REFERENCES

D-DEZ-PRO-006 ICC Protocol for Pre-fixed Ocular Tissue

### D. MATERIALS, EQUIPMENT AND MEDIA

Water bath (Thermo Fisher) Centrifuge (Thermo Fisher) Rotating shaker (Forma Scientific) -20C freezer (Thermo Fisher)

#### Polyvinyl alcohol (PVA) with DABCO

1,4-Diazabicyclo[2.2.2]octane (DABCO), antifade reagent (Sigma, order # 290734-100ML)

Polyvinyl alcohol (PVA), self-hardening reagent (Sigma, order # 341584-25G)

Glycerol (University of Iowa Biochemistry Stores)

Molecular grade water (Life Technologies, Order # 10977-023)

Tris-HCL (University of Iowa Biochemistry Stores)

DAPI (Sigma, Order # 32670-5MG-F)

A similar product can be obtained ready to use from Sigma-Fluka (Polyvinyl Alcohol Mounting Mediaum with DABCO, antifading, Order # 10981)

#### E. DAY ONE

- 1. Add 4.8 g of polyvinyl alcohol (PVA) to 12 g of glycerol and mix well.
- 2. Add 12 ml of molecular grade water and leave it on a rotator at room temperature overnight.

#### F. DAY TWO

- 1. Add 24 ml of 0.2M Tris-HCL at pH 8-8.5.
- 2. Heat in a water bath to 50°C while mixing for 30 minutes.
- 3. Add 1.25 g of DABCO and mix well.
- 4. Centrifuge at room temperature at 200g for 5 minutes.

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- 5. If desired add DAPI at 2 ug/ml.
- 6. Aliquot the supernatant (we use 1 ml aliquots which is enough for 15-20 slides) and store at -20°C.
- 7. Since it polymerizes upon contact with air you should not refreeze the aliquots. Thaw it just before use and throw away what you do not use.

## G. HISTORY

Effective Date	Revision	Change
07 Apr 2015	0	PM-18 Rev. #3