# Preparation of Propolyene Glycol and Sucrose for Controlled Rate Freezing

## 1.0 Equipment

**1.1** Analytical balance

**1.2** Spatula

**1.3** 1000µl Gilson pipette

**1.4** 10ml Gilson pipette

**1.5** Test tube holder

**1.6** Safety glasses

**1.7** 10ml Volumetric Flask with lid

## 2.0 Supplies

**2.1** 14ml Falcon plastic tubes

**2.2** 0.2µm syringe filters

**2.3** 10ml plastic disposable syringes

**2.4** 100-1000µl pipette tips

**2.5** 10ml pipette tips

**2.6** Propylene Glycol (1,2-Propanediol)

**2.7** Sucrose

**2.8** Weighing paper

**2.9** Purified water

**2.10** Filtered M2 medium

**2.11** Tissues

**2.12** Parafilm

**2.13** 60ml sterile media bottle

## 3.0 Procedure

### 3.1 General Information

3.1.1 Safety glasses must be worn at all times by the person preparing the media, and by those working in the immediate area.

### 3.2 Sucrose Preparation

3.2.1 Using a 10ml Gilson pipette add 5ml of M2 to a 60ml sterile media bottle.

3.2.2 Weigh out 3.42g of sucrose onto a weighing paper on the analytical balance. Use a spatula that has been rinsed in purified water and dried with a tissue prior to use.

3.2.3 Carefully pour the sucrose into the media bottle containing M2 to avoid spilling any granules.

3.2.4 Place on lid and invert the media bottle gently to mix the solution until the sucrose is fully dissolved. This may take some time.

3.2.5 Once the sucrose has fully dissolved, transfer the solution into a 10ml volumetric flask.

3.2.6 Rinse the media bottle 2-3 times with M2 and use this to make the sucrose solution up to 10ml until the bottom of the meniscus rests on the line. The last ~1ml can be added in smaller volumes using a 1000µl Gilson pipette to ensure the solution does not go over the line.

3.2.7 Cover the top of the flask with Parafilm and invert 5-6 times to mix.

3.2.8 Filter into a clean 14ml Falcon tube using a 0.2μm filter with a 10ml syringe. Make sure the filter does not come into contact with any contaminating surfaces.

3.2.9 Store in a labelled and dated 14ml falcon tube at 4°C for up to 7 days.

**NOTE:** Label the lid of the Falcon tube with an 'S' to ensure the lid does not get mixed up with other solutions.

## 3.3 Propylene Glycol Preparation

3.3.1 Stand a 60ml sterile media bottle on an analytical balance.

3.3.2 Using a 1000μl Gilson pipette, weigh out 1.14g of Propylene Glycol into the 60ml sterile media bottle.

**NOTE:** Care must be taken to add the Propylene Glycol slowly, due to its viscosity. The solution should be allowed to drip down the pipette tip before dispersal.

3.3.3 Use a 10ml Gilson pipette to add 5ml M2 into the media bottle containing Propylene Glycol.

3.3.4 Place on lid and invert the bottle gently to mix the solution until it is fully mixed.

3.3.5 Once the solution is fully mixed, transfer the solution into a 10ml volumetric flask.

3.3.6 Rinse the media bottle 2-3 times with M2 and use this to make the Propylene Glycol solution up to 10ml until the bottom of the meniscus rests on the line. The last ~1ml can be added in smaller volumes using a 1000μl Gilson pipette to ensure the solution does not go over the line.

3.3.7 Cover the top of the flask with Parafilm and invert 5-6 times to mix.

3.3.8 Filter into a 14ml Falcon tube using a 0.2µm filter with a 10ml syringe. Make sure the filter does not come into contact with any contaminating surfaces.

3.3.9 Store in a labelled and dated 14ml falcon tube at 4°C for up to 7 days.

**NOTE:** Label the lid of the falcon tube with a 'P' to ensure the lid does not get mixed up with other solutions.

## 3.4 Testing the Propylene Glycol

3.4.1 The concentrated Propylene Glycol has a shelf life of 6 months once opened.

3.4.2 Each bottle of Propylene Glycol (Pro) should be tested before use. The Pro can either be tested using the controlled rate freezer (Biocool), or by testing the vitrification media as the Pro forms part of the DAP213.

3.4.3 A Pro test requires embryos (from a passed FZ session or fresh embryos), tested dishes and tested oil to be used. The old batch of media should be used as a control.

3.4.4 Freeze half of the embryos using the batch of Pro to be tested and freeze the other half of the embryos using a proven batch of Pro, ensuring the tested and untested batches of Pro are kept separate.

3.4.5 If using frozen embryos make sure they are from a tested FZ session and thaw the embryos according the appropriate thawing method depending on the freezing method. Ensure safety glasses are worn.

3.4.6 Once the embryos have been frozen, thaw them making sure at all times not to mix the batches.

3.4.7 Culture the embryos and check their development daily until they develop to blastocyst stage.