

Preparation of KSOM

1.0 Equipment:

- 1.1** Heat-stirrer CB162 Magnetic stirrer
- 1.2** Magnetic flea
- 1.3** 1L beaker
- 1.4** Analytical Balance
- 1.5** Electric filter pump
- 1.6** Gilson pipette 200
- 1.7** Pipette boy
- 1.8** 500ml Measuring cylinder x2
- 1.9** Squeezy bottle labelled Embryo water
- 1.10** Safety glasses
- 1.11** Test tube holder
- 1.12** -20⁰C freezer
- 1.13** 4⁰C Fridge

2.0 Supplies:

- 2.1** Weighing boats
- 2.2** Spatulas
- 2.3** 10ml pipette tip
- 2.4** 60ml capped bottles
- 2.5** 1ml pipette tips
- 2.6** 14ml falcon tubes
- 2.7** Permanent marker pen

2.8 70% Ethanol

2.9 15mm One well dishes

3.0 Reagents for Lab made KSOM:

3.1 NaCl (Sodium chloride)

3.2 KCl (potassium chloride)

3.3 KH₂PO₄ (potassium phosphate monobasic)

3.4 MgSO₄ 7H₂O (Magnesium sulfate)

3.5 CaCl₂ 2H₂O (Calcium chloride dihydrate)

3.6 NaHCO₃ (Sodium bicarbonate)

3.7 Glucose

3.8 Na-Pyruvate

3.9 DL-Lactic Acid, sodium salt

3.10 10mM EDTA

3.11 Streptomycin

3.12 Penicillin

3.13 0.5% phenol red

3.14 L-Glutamine

3.15 MEM Essential Amino Acids

3.16 MEM Non-essential AA

3.17 BSA

3.18 Embryo water

4.0 Procedure:

4.1 General Information

- 4.1.1 When making KSOM, use the attached tick sheet to ensure correct reagents and amounts added (see Appendix 1).
- 4.1.2 Use appropriate size spatula and boat for each reagent. Use a new spatula and boat for each reagent. The spatulas can be cleaned and autoclaved at the end of the procedure so they can be re-used.
- 4.1.3 Safety glasses, gloves and mask should be worn at all times when handling the reagents.
- 4.1.4 Each new batch of KSOM requires Quality testing. Batch numbers (date) can be found on the bottle label. Only KSOM that has been previously batch tested for quality control should be used (See 4.3 KSOM Quality control).
- 4.1.5 KSOM must be stored in the freezer and slow defrosted in the fridge overnight until fully thawed.
- 4.1.6 The KSOM can be used for up to two weeks of the thawed date.

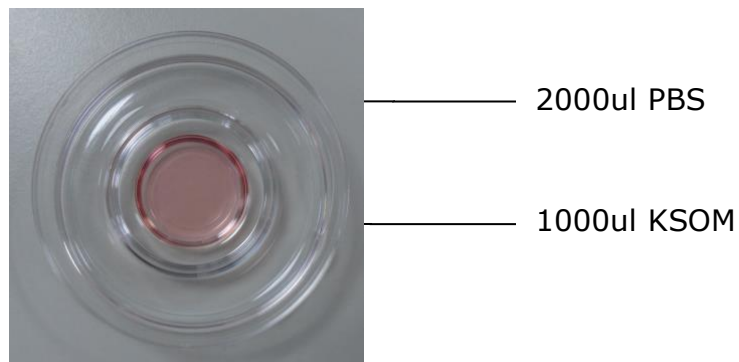
4.2 Preparation of media

- 4.2.1 Place a 1L beaker on the heat stirrer. Pour 200-300 ml of embryo water into a 1L beaker. Decant the remaining embryo water into the squeeze bottle marked 'embryo water'.
- 4.2.2 Place the magnetic flea in the bottom of the beaker and switch the stirrer on. Begin to stir at setting 2, without heat.
- 4.2.3 Place a weighing boat on the scales, ensuring it is reset to 0g.
- 4.2.4 Add the date to the top of the reagents tick sheet; this will also be your batch number. Work down the reagents tick sheet collecting each reagent when needed and adding to the beaker. Make a note of how much has been added next to each reagent on the sheet (see Appendix 1).

- 4.2.5 Select suitable spatulas and boats according to which reagent you are adding. Use embryo water to wash off any residual reagents left on the boat. A new spatula and boat must be used for each reagent. Gilson pipette and tips should be used for the liquid reagents.
- 4.2.6 Once there is enough liquid in the beaker, increase the heat stirrer speed to 3.
- 4.2.7 When all the reagents have been added to the beaker and mixed, turn off the stirrer. Take the 2x500ml measuring cylinders and divide the mixture between them.
- 4.2.8 Top up each cylinder to 500ml with embryo water to make 1L.
- 4.2.9 Pour the mixture back into the beaker, reset the stirrer to 3 and continue mixing for 1min.
- 4.2.10 Set up the 1L filter pump. Filter the solution through a Corning 1000ml filter unit with the electric pump.
- 4.2.11 Once filtered, aliquot the media into 60ml capped bottles. Label the bottles with the date and contents, e.g. 29/10/2018 KSOM. The date on the bottle will be the batch number.
- 4.2.12 Place bottles in the freezer. Each batch must be tested before use (see 4.3). Lab made KSOM can be stored in the freezer for up to one year from the date made.

4.3 KSOM Quality control

- 4.3.1 Take required bottle of KSOM from the freezer. Allow to thaw in the fridge overnight to ensure a slow thaw.
- 4.3.2 Once the KSOM has thawed take two 15ml 1 well culture dish and pipette 1ml of KSOM into the well. Pipette 2ml of PBS around the outside of the well (Picture 1). Place this into the incubator and allow to equilibrate for a minimum of 7 hours. This can be done first thing in the morning to use in the afternoon, but preferably the night before.

Picture 1

- 4.3.3 Collect fertile one cell embryos and place into the KSOM dish. Record the following details on the dish e.g. KSOM QC, 30/08/18, Batch no.20/03/18, 30 1cell. Place the dish with the embryos back into the incubator.
- 4.3.4 Check the following day and record the number of 2 cells that have developed overnight.
- 4.3.5 80% overnight development is required for the KSOM to pass the QC. If 80% is not achieved repeat from 4.3.1 using a new bottle of KSOM from the same batch. If it fails for a second time this batch may need to be discarded.

4.4 Aliquoting KSOM

- 4.4.1 Remove frozen KSOM from the freezer the night before intended use and store in the fridge. In an emergency KSOM can be thawed on the bench, but only as a last resort should this be done.
- 4.4.2 Check the use by date and batch number. Ensure that the batch has been tested.
- 4.4.3 Take the defrosted unopened bottle of KSOM from the fridge and decant 10ml of KSOM into 5 Falcon tubes.
- 4.4.4 Label each tube, using a permanent marker, with KSOM and the date e.g. KSOM 01/05, use within two weeks but preferably only use for one week.
- 4.4.5 Store in the fridge until needed. Place one falcon tube of KSOM in the incubator overnight, when possible, to allow equilibrium of KSOM. This can also be done first thing on the morning of use.

5.0 Appendix 1

Reagent	Location	mg/ml	Amount added to batch
Sodium Chloride	MI cupboard	5.55g	
Potassium Chloride	MI cupboard	0.185g	
Potassium Phosphate Monobasic	MI cupboard	0.0475g	
Magnesium Sulfate	MI cupboard	0.0495g	
Calcium Chloride Dihydrate	MI cupboard	0.25g	
Sodium Bicarbonate	MI cupboard	2.10g	
Glucose	MI cupboard	0.036g	
10mM EDTA	MI cupboard	1ml	
Phenol red	MI cupboard	1ml	
Sodium DL-lactate Solution	MI fridge	1.74ml	
Streptomycin Sulfate Salt	MI fridge	0.05g	
Penicillin G Potassium Salt	MI fridge	0.06g	
Sodium Pyruvate	MI fridge	0.022g	
L-Glutamine	MI fridge	0.146g	
MEM Amino Acid	MI fridge	10ml	
MEM Non-essential Amino Acid Solution	MI fridge	5ml	
Bovine Serum Albumin	MI fridge	1g	

Date/batch number made: