Aberdeen Primary culture of fish macrophage cells from Head Kidney

Reagent	catalogue no	supplier
L15	11415049	Gibco
FBS	FB100/500	Biosera
Heparin	H3393-10KU	Sigma
Pen/Strep	P4333-100ML	Sigma
Percoll	10232919	GE Health care
10xHBSS	H4641-500ML	Sigma
Na_2Co_3	S7795-500G	Sigma
Molecular grade water	7732-18-5	Sigma
Sterile Syringe	Z683620	Sigma
Sterile 100um nylon mash	CC8111-0102	Starlab

Preparation before cell extraction
Fish dissection
One polystyrene box with ice
Balance
Scalpels
Little tweezers and scissors
50ml tubes
Marker, pen and notepad
Absorbent paper

Prepare cell extraction media using the recipe below and put 20ml of the media in a 50ml tube. Put the tube on ice and take it with you to wet lab downstairs to put the head kidney when dissecting the fish

Fish Dissection

Euthanise fish with 2-phenoxyethanol and kill the fish by stabbing on the back of head using a sharp scissor. Extract the head kidney and put it in a 50ml tube with around 20ml extraction media in it

Recipes for making medias

Extraction media (500ml)	
Reagent	**volume**
L15	490 ml
2% FBS	9.95 ml
0.1% heparin	500 ul

Washing media (50ml)	
Reagent	**volume**
L15	49.9ml
0.1% FBS	50 ul
0.1% heparin	50 ul
Growth media (500ml)	
Reagent	**volume**
L15	445 ml
10% FBS	50 ml
1%Pen/ Strep	5 ml
51% Percoll (50ml)	
H2O	18.9 ml
Percoll	25.5 ml
10xHBSS	5 ml
Sodium bicarbonate (5M)	357 ul
Heparin (1ul/U)	50 ul

Procedure:

- Take a 100um nylon mash and fit it inti a new 50ml tube.
- Take also a new 50ml tube and fill it with extraction media (you will need these to mash up the cells)
- Uisng a sterile pipette, take the head kidney from the extraction media and place it on nylon mash
- Gently squeeze the head kidney though 100um nylon mash using a sterile spatula or back of a sterile syringe and plenty of extraction media, this may take 10 minutes to do as need to be gentle, head kidney tissue will break up quite easily. **Make up to 40ml of cells and extraction media**

Separate macrophages from erythrocytes through a percoll gradient

Put 5ml of 51% percoll into 50 ml falcon tube, VERY gently layer 20ml of cell suspension on top of this, the cell should float as the L15 is less dense than the percoll.

Spin 30 min at 400g – programme no. 29 on centrifuge

Cells at interface should be macrophages, RBCs should have pelleted.
Carefully remove interface cells to new tube.
Make up to 20 ml with wash buffer

Spin 10 min 400g – programme no. 30

Cells will pellet loosely at the bottom
Pour way the growth media, carefully making sure not to throw away the pellet

Resuspend in 20ml growth media

Count cells
try and get conc to $1x10^6$-$1x10^7$ ml
Put about 20 ml vol into a 80cm flask.?