Protocol for isolation of Endothelial cells from embryos

1) Use sterile conditions; autoclave forceps. Work in hood.

2) Coat plates with .1% gelatin for at least 30min in 37° and allow to air dry (if sorting)

3) Add 10ml of DMEM/antibiotics to 10cm dish

4) Transfer embryos to dish and move to hood

5) Remove media, add more and rinse embryos (dissect out embryos from placenta)

6) Add 1mg/ml collagenase in media no serum (6ml) to 15ml tube per embryo

7) Pipette up and down, try to crush embryo as best as possible

8) Rotate at 37°C incubator for 15min

9) Use 10ml pipette to resuspend well

10) Leave in 37°C for additional 15min

11) Resuspend well with 25ml pipet (should be few clumps)

12) Add 7ml of 10% FBS/media and mix well

13) Spin 5 min at 1900 RPM and resuspend pellet in 10ml lysis buffer

14) Incubate 3 min at RT

15) Fill up tube with DMEM/serum and spin

16) Resuspend cells

17) Put through a 40um filter

18) After cells have gone through filter, count

19) Spin down for 6min at 2000

20) Remove media and add as much media as needed
**EC media MAEC**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
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<tbody>
<tr>
<td>DMEM</td>
<td>500ml</td>
</tr>
<tr>
<td>20% FBS</td>
<td>100ml</td>
</tr>
<tr>
<td>1X NEAA</td>
<td>5ml</td>
</tr>
<tr>
<td>1X L-glutamine</td>
<td>5ml</td>
</tr>
<tr>
<td>1x Pen/Strep</td>
<td>5ml</td>
</tr>
<tr>
<td>50ug/ml gentamicin</td>
<td>500ul</td>
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</tbody>
</table>

**Add following to stock:**

- Heparin (10ug/ul) add 50ul for 50ml
- Endothelial cell growth factor (5mg/ml) add 1ml for 50ml
- cAMP (25mg/ml) add 50ul for 50ml
- Retinoic acid (1mg/ml) add 15ul doe 50ml

**Lysis Buffer (10X)**

- Ammchl 8.29g
- K(HCO₃)₂ 1g
- EDTA pH 8.037g
- Add H₂O to 100ml