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

DMEM	500ml
20% FBS	100ml
1X NEAA	5ml
1X L-glutamine	5ml
1x Pen/Strep	5ml
50ug/ml gentamicin	500ul

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
$K(HCO_3)_2$ 1g
EDTA pH 8 .037g
Add H_20 to 100ml