

Olfactory Bulb Explant Cultures

PART I

(Start this culture about 7-10 days before the experiment)

A) 293 cells from stock

- Thaw a vial of cells at 37°C
- Re-suspend in 10 mL of 293 growth medium
- Centrifuge at 1,000 rpm for 5 min.
- Re-suspend pellet in 10 mL of 293 growth medium and plate cells in 100 mm petri dishes
- Incubate at 37°C and 5% CO₂ for 3 days

1.) After 3 days:

- Aspirate the old medium
- Wash cells twice with 10 mL of 1X PBS / 100 mm dish
- Add 1.5 mL of Trypsin/EDTA solution and incubate for 3-5min at 37°C
- Add 10 mL of 293 growth medium, re-suspend and transfer to a 15 mL tube
- Spin for 5 min at 1,000 rpm at room temperature
- Aspirate medium and re-suspend in 293 growth medium
- Count cells, for transfection, plate at 2.5 mL x 10⁵ (in 2 mL of growth medium) per well in poly-D-lysine coated-six well plates, incubate for 2 days, or until confluent.

B) 293 cell transfection

- Per well to be transfected prepare:
 - 1.5 mL eppendorf with 12.5 µL of lipofectamine plus 350 µL of DMEM
 - 1.5 mL eppendorf with 5 µg of the DNA of interest plus 350 µL of DMEM
- Incubate each tube for 5 min at room temperature
- Mix the 2 tubes and incubate for 20 min at room temperature
- Aspirate old medium from plate and add the content of the tube gently to the cells
- Add 293 growth media up to 2.5 mL
- Incubate at 37°C and 5% CO₂ overnight

C) 293 cells aggregates

(Day of experiment)

- Wash cells twice with PBS
- Add trypsin/EDTA for 5 min (0.5 mL / 6 well plate)
- Re-suspend in 10 mL of 293 growth medium and transfer to a 15 mL tube
- Spin at 1,000 rpm for 5 minutes
- Re-suspend cells in 10 mL of DMEM with 1% FBS and 1% Pen/Strep
- Spin at 1,000 rpm for 5 minutes
- Re-suspend in 30 µL of collagen-matrigel solution (always on ice!!!)
- Make 8 µL droplets of cell suspension on the cover of a 3 cm dish
- Put 2 mL of 293 growth medium in a 3 cm dish and invert cover on top of it
- Incubate at 37°C for 2-3 hours (drops will solidify)
- Carefully invert cover back and refresh medium when the medium starts to turn yellow
- Incubate at 37°C until use (up to 6 hrs total).
- When ready to use, chop each drop in pieces of about 300-400 µm. Keep them in 293 growth medium until plating.

PART II

(Day of experiment)

A) OE-OB-VNO dissection

- Use E15-E17 embryos (in older embryos the survival decreases dramatically under our culture conditions)
- After C-section, place clean embryos in cold L15 / 5%FBS (storage media) on ice.
- For dissection place each head in L15 (dissection media) in a clean plate and store a piece of tail for genotyping.
- During the dissection keep the tissue covered with media.
- Under microscope, open the skull and dissect first the OBs (place them in clean plate with L15 / 5%FBS on ice).
- With forceps, make a cut at the orbital level downward (in between the face and the brain), and keep the rostral part of the head.
- Remove the inferior jaw and place the snout with the palatine facing up.
- Open the palatine, and the ventral part of the septum including the VNO will be exposed.
- Clean away the excess of tissue in order to get at all of the septum.
- Remove the VNO (small and sausage-shaped) and put into a new plate with storage media on ice.
- OE at this age is a very thin transparent lamina in both sides of the septum. Place the septum in storage media and remove OE carefully with fine forceps trying not to remove the lamina propria (pink tissue).

B) Explant preparation:

- **OE/OB:** After dissection of all the embryos, chop OE or OB tissues with fine forceps into pieces of about 300 μm . Keep them in storage media on ice until plating. When plating the OE, choose only the pieces of tissue that look transparent (to ensure that the OE tissue is clean).
- **VNO:** After the dissection, place individual VNOs in an eppendorf, add 500 μl of Pancreatin 10X:Trypsin-EDTA 0.25% (1:1) and incubate on ice for 30 min. Thereafter, remove the liquid and incubate the VNO for 45 min in 500 μl of L15:FBS (1:1). When ready, place each VNO in a clean dish in storage media and separate the vomeronasal epithelia (transparent) from the surrounding mesenchymal tissue. Chop with fine forceps to about 300 μm pieces. Keep on ice until plating.

C) Plating on Collagen-Matrigel Matrix:

- Prepare collagen-matrigel solution and always keep it on ice or it will become solid!!!
- Make 20 μl flat (!!) droplets of collagen solution per well in 4 well plate. Avoid air bubbles!
- Let stand in incubator @ 37°C for 30-45 minutes (no more, gets too dry).
- Using small tip, pipette a piece of cell aggregate and place it on top of collagen droplet.
- Put 2 or 3 pieces of tissue (OE/OB/VNO) next to cell aggregate and add 20 μl of collagen-matrigel on top.
- Position the explants close to the cell aggregate (ideal distance should be 1-2 explant diameter) with a sterile needle.
- Let stand @ 37°C for 45 minutes, or until solid.
- Carefully add NB/B27 media on top of the explant (400 μl /well).

- Culture for 2-4 days. Change medium every other day.

Media and Solutions:

293 cells Growth Medium:

100ml DMEM 1X
10ml FBS
1ml Pen/Strept
Filter through 0.22 μ m filter system and store at 4°C

Collagen Stock Solution:

Prepare a solution of collagen 2.5 mg/mL with cold 0.1X DME
Keep sterile @ 4°C

0.1X DME:

0.95 g MEM
960 mL milli-Q water
pH to 4 with 10N HCl
add 10 mL penicillin-streptomycin
add milli-Q water up to 1 L
Keep at 4°C

Collagen-Matrigel Solution:

(enough for one 4 well plate):

Cool eppendorfs on ice and put all components on ice

Freshly prepare:

160 μ L Collagen stock solution
20 μ L Matrigel
20 μ L HBSS 10X
2 μ L of 1 M sterile Na₂CO₃
Mix gently (turns dark pink) and keep on ice all the time

NB/B27 medium:

Defrost B27 and Glutamine in 37°C water bath
Remove 15 mL from 500 mL Neurobasal bottle
Add 10 mL B27, 5 mL of 200 mM Glutamine (2 mM final)
Add 5 mL Pen/Strep
Filter through 0.22 μ m filter and store at 4°C

Product Ordering Information:

B27 supplement, 17504044, Invitrogen
Collagen, Type I; from Rat Tail, C7661, Sigma
DMEM (1X with 4.5 g/L glucose, L-glutamine & sodium pyruvate), 10-013-CV, Cellgro
HBSS (10X), H1461, Sigma
FBS (characterized), SH30071.03, Hyclone
Leibovitz 's L15 Medium (1X), 11415, GIBCO
Lipofectamine™ 2000, 11668-027, Invitrogen, -
Matrigel™ 356234, Becton Dickson Biosciences
MEM (with Earle's salts and L-glutamine, without sodium bicarbonate), 61100-012, GIBCO
Neurobasal media, 21203-049, Invitrogen,
Pancreatin (10X), 02-0036DG, GIBCO
Penicillin-Streptomycin, 15140-015, GIBCO
NUNC 4 well plates, 176740, NUNC
Poly-D-lysine , P0899, Sigma
Trypsin/EDTA (0.25%), 25200056, Invitrogen