**METHOD TO GROW CHICKEN NEUROECTODERM EXPLANTS IN VITRO**

Collagen preparation (keep collagen over ice at all times)

1. To 2.16 µl of 2.5M NaOH add 50 µl of 10x MEM.

 Mix by pipetting gently 🡪 Solution: PINK

2. Add mixture to a collagen aliquot (450 µl at 3.1 mg/ml).

 Mix by pipetting gently 🡪 Solution: APRICOT

3. Add 22 µl Bicarbonate solution (7.5% BIC)

 Mix by pipetting gently 🡪 Solution: PALE PINK

Collagen bedding

4. Spread 16µl of collagen solution per well, and spread with tip of pipette.

5. Dry collagen by placing covered dish in incubator at 37°C for 30 minutes.

 🡪 Solution: MILKY WHITE

Collagen embedding of explants

6. Transfer explants to 50 µl drop of collagen and rinse carefully not to make bubbles.

7. Transfer explant to 20 µl drop of collagen.

8. Transfer explant with the 20 µl of collagen to the dried collagen bead in well.

9. Position explant in collagen using forceps.

10. Dry collagen in incubator at 37°C for 60 minutes.

Media

11. Add 650 µl of Serum Free OPTI MEM-Media supplemented with N2-supplement, fibronectin, and any of the following factors. Grow explants in incubator at 37°C and 5% CO2 until needed.

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| --- | --- | --- | --- | --- | --- | --- |
| **Reagent** | **Stock conc.** | **Final conc.** | **1 well** | **2 wells** | **4 wells** | **8 wells** |
| **Media** | 1x | 1x | 0.65 ml | 1.3 ml | 2.6 ml | 5.2 ml |
| **FGF** | 100 µg/ml | 0.03 µg/ml | 0.78 µl | 1.56 µl | 3.12 µl | 6.24 µl |
| **Wnt3a** | 40 µg/ml | 0.15 µg/ml | 2.437 µl | 4.875 µl | 9.75 µl | 19.5 µl |
| **RA** | 10 mM | 0.01 mM | 0.65 µl | 1.3 µl | 2.6 µl | 5.2 µl |

References

-Alekseenko, Andersson and Dias (2015) Bio-Protocols 5:1.

-Nordstrom, Maier, Jessell and Edlun (2006) PLoS Biology 4:e252.

\*\*\* St 4 explants grown for 15 hours express Cdx and Hox genes

\*\*\*Wnt+RA🡪 cHB, Wnt+RA+FGF🡪 rSC, Wnt+FGF🡪 cSC