

Primary Mouse (embryonic or postnatal) Cortical Neuron Culture--Dissociated via Papain

- **Papain Kit Storage:**
 - Store kit at 4°C
 - Keep papain and DNase vials in a dessicator
 - Reconstitute ovomucoid inhibitor in 37 mL of EBSS
 - Make 5 X 7.5 mL aliquots and store in dessicator as well
- **Poly-D-Lysine (PDL) coating:**
 - **To prepare 1X stock:**
 - Add 333.35 mL of sterile ddH₂O to 50 mg of PDL.
 - Mix and filter thru 0.22 µm filter.
 - Aliquot and store @ -20°C.
 - Use within 1-2 weeks
 - Only freeze / thaw once!
 - **To coat plates:**
 - Thaw PDL at 37°C
 - Filter thru 0.22 µm filter
 - Coat with the following volumes:

• 96 well plates	50-60 µL/well
• 4 well slides	300 µL/well
• 24 well plates	500 µL/well
• 6 well plates	2 mL/well
• T25 flasks	4 mL/flask
 - Incubate at 37°C for at least 2 hr (best if left on overnight)
 - 1-2 hours prior to the prep remove plates from incubator and wash 2 times with sterile ddH₂O.
 - Allow plates to dry completely in hood.
- **Dissection/Enzyme Preparation:**
 - Warm dissection media and place in appropriate dishes.
 - Warm plating media in 37°C H₂O bath.
 - Sacrifice the mother by cervical dislocation.
 - Spray lower abdomen with 70% EtOH and cut through skin and muscle with a pair of scissors exposing the uterus, intestines and embryos.
 - Cut embryos from amniotic sac.
 - Remove brain from skull.
 - Dissect cortex from each lobe.
 - Prepare papain when prep is 75% complete as follows:
 - Reconstitute papain in 5mL of Earl's Balanced Salt Solution (EBSS).
 - Place in 37°C incubator for about ten minutes
 - While papain is equilibrating, finish the dissection and place both cortices from one pup into a 15 mL conical tube filled with EBSS.
 - You should have one tube per pup (Make sure to keep the tubes well labeled as genotyping will be done later).
- **Plating Procedure:**
 - Reconstitute DNase in 500 µL of EBSS.
 - Add 125 µL of DNase to 2.5 mL of papain solution.
 - Remove EBSS media from cortices (as much as possible via glass pipette) and add the papain/DNase solution to the tissue.
 - Place the tissue/enzyme in a 37°C H₂O bath and allow to incubate as follows:

- 1 embryonic litter = 8 minutes
 - 1 post-natal litter = 12 minutes
- Invert the tissue/enzyme tube 3 times every four minutes.
- After the incubation is complete, triturate tissue with a 10 mL pipette (13 times) followed by a flamed tip glass pipette (13 times).
- Place tube in rack and allow any remaining chunks of tissue to settle to the bottom of the tube.
- Once the tissue has settled, remove the cloudy supernatant (via glass pipette) and place into a new 15 mL conical tube.
- Centrifuge the supernatant at 2000 rpm for 5 minutes.
- While centrifuging, prepare re-suspension media as follows:
 - Place 1.35 mL of EBSS in a 15 mL conical tube
 - Add 150 μ L of albumin-ovomuroid inhibitor to the tube
 - Add 75 μ L of DNase to the tube
- After the 5 minute spin is complete, remove supernatant and re-suspend the cell pellet with the above re-suspension media...
 - Use a *flamed tip* glass pipette to re-suspend the cells (Triturate ~ 7 times).
 - If you feel you still have too many tissue chunks, you can filter through a 70 and / or 40 micron cell strainer.
- Prepare a continuous density gradient as follows:
 - Add 2.5 mL of albumin inhibitor solution to a new 15 mL conical tube.
 - **Carefully** layer the cell suspension unto the top of the 2.5 mL of inhibitor solution.
 - Add the cell suspension very slowly!
 - You should begin to see three layers appear:
 - Cloudy pink on top
 - Clear, lighter pink in the middle
 - Yellow on the bottom
- Centrifuge the gradient solution at 1000 rpm for 5 minutes.
- Remove the supernatant and re-suspend the pellet as follows via a *flamed tip* glass pipette:
 - 1 mouse pup = re-suspend in 5 mL of NB/B27
- Count cells on hemacytometer by diluting 20 μ L of cell suspension in 180 μ L of plating media (10x dilution)
 - Dilute cells to 0.5×10^6 cells/mL
- Plate cells using the following volumes:

▪ 96 well plates	100 μ L/well
▪ 4 chamber slides	500 μ L/chamber
▪ 24 well plates	1 mL/well
▪ 12 well plates	2 mL/well
▪ 6 well plates	4 mL/well
▪ T25 flasks	10 mL/flask
- **Feeding/Inhibition:**
 - Feed cells every 3 days.
 - Remove 50% of the media and replace with fresh feeding media.
 - Never do a 100% change!
 - Account for evaporation when keeping cells for long periods.
 - ie... for a 96 well plate, remove 40 μ L and add back 60 μ L
 - The initial feeding will be on DIV3 and will be the “inhibitory” feeding with Ara-C.

- The final concentration of Ara-C on this feeding is 1 μ M.
 - Aliquots of 10 mM Ara-C in ddH₂O are stored @ -20°C
 - 20 μ l of 10 mM Ara-C is added to 100 mL of feeding media = 2 μ M
 - 1/2 change of media = final of 1 μ M.

Solutions:

- NB/B27 Plating and Feeding Media
 - Defrost B27 and Glutamine in 37°C H₂O bath
 - From 500 mL Neurobasal bottle, remove 15 mL
 - Add back:
 - 10 mL of B27
 - 5 mL of 200 mM Glutamine (2 mM final)
 - if necessary, may add 0.5-1.0% pen/strep
 - Filter through 0.22 μ m filter system, label and store @ 4°C

Product Ordering Information:

Papain Kit, LK003153, Worthington

70 micron cell strainers, Becton Dickson, 08-771-2

Hanks Balanced Salt Solution (w/o phenol red), 114-062-101, Quality Biologics

Ara-C, C6645, Sigma

B27 Supplement, 17504044, Invitrogen

DNase, D4513, Sigma

FBS (Characterized), SH30071.03, HyClone

L-glutamine, 25030-081, Invitrogen

Neurobasal Media, 21103-049, Invitrogen

6 well NUNC plates, 62407-073, VWR

96 well NUNC plates, 25382-342, VWR

Poly-D-Lysine, P0899, Sigma

Tweezers, RS-5005, Roboz

Forceps, 11251-35, FST

Spring scissors, 15023-10, FST