NEUROMICS

Frozen E18 Primary Rat Cells from Hippocampus

Thaw and Culture Protocol

Catalog Number: PC35111

Components : •**Approximately 1 x 10^6 (1 million) .**
*Neuromics frozen embryonic rat hippocampal neurons are guaranteed to produce 20% survival (100*live / (live+dead) cells) after thawing and 4 days in culture.*

Shipping/Storage: Frozen neurons are shipped on dry ice. Upon receipt, they must be stored at -70 degrees Celsius or below.

Application Notes

Materials required but not supplied (recommended examples):

a. Substrate: glass coverslips-Neuromics #M3610 or # 63-3031 (www.carolina.com); or 24-well plastic-Corning #3526.
b. Medium-NbActiv4™ (Neuromics#M36107) or Neurobasal, 50x B27, 0.5 mM glutamine or Glutamax (all Invitrogen).
c. 15 ml sterile polystyrene or PET centrifuge tube (Corning #430055).
d. 70% ethanol.
e. Water bath, 37ºC .
f. Hemacytometer (VWR #15170-079), trypan blue (Sigma #T8154) and inverted phase contrast microscope.
g. Sterile culture-grade water (e.g. 18 Megohm).
h. 5% CO_2, 37ºC humidified incubator (Even better, 5% CO_2, 9% O_2, eg. Forma 3130).
i. Sterile hood with vacuum source.
j. Poly-D-lysine hydrobromide (Sigma P6407) dissolve with sterile 18 MΩ H_2O to 100µg/ml. Store in PET or polystyrene tube (not polypropylene) at -20ºC. Thawed no more than 2 times

Preparation of Substrates:

Note: Neurons are adhesion dependent. Surfaces must be coated with adhesive >1-20 hr. before thawing cells.
Prepared Poly-D-Lysine coated coverslips: #M3610 are available from Neuromics.

k. In sterile hood, coat the sterile cell culture polystyrene or glass with sterile Poly-D-Lysine (0.15ml/cm^2, 50µg/ml). Let sit for 1 to 20 hrs. Do not let dry.
l. Aspirate with sterile stip.
m. Rinse one time with sterile water and let substrate dry in sterile hood (can be stored at 4ºC for a few days-best when used immediately).

Preparation of medium

(about 0.25 ml/cm^2 substrate) (can be stored at 4ºC for 1 week)

Growth medium: NbActiv4™ or Neurobasal, 2% B-27, 0.5mM Glutamax. Bring to room temperature.

Plating frozen - thawed neurons

1. Thaw one tube at a time (leave others frozen) for a minimum amount of time in 37° C waterbath (target 3 min). Remove immediately when thawed. Do not vortex or shake vigorously. Wipe outside of cryovial with 70% ethanol.

2. Transfer 0.85 ml to a 50 ml centrifuge tube without creating bubbles.

3. Add 7.65 ml room temperature medium dropwise over a period of 2 minutes. Mix gently as you add medium. Gently mix by inversion two times.

4. Immediately plate 0.2 ml/cm^2 substrate (range 0.15-0.5 ml/cm^2) or 2 ml per dish containing 3 precoated slips. Gently mix cell suspension every 2 minutes in between aliquots.

5. Incubate plated cells 37°C, 5% CO_2, 9% O_2, (ambient oxygen will work also) . Also place more medium in incubator with loose cap to equilibrate pH.

6. Mix leftover cells from step 4 and aliquot 30 µl of cells into 30 µl of trypan blue. Count cells with hemacytometer (10-10x fields).

7. After 0.7-3 hrs incubation, remove culture from incubator for medium exchange. Fill pipet with fresh medium equilibrated in 37°C, 5% CO_2 , 0.2 or 0.4 ml fresh medium/cm^2 (for 35 mm dish use 2.0 mL). Drain and aspirate medium over cells and immediately add fresh medium. Be careful not to let cells dry out. Be careful to add medium gently from side so that adherent cells are not disturbed. Quickly observe phase bright adherent cells with 10 or 20x objective mag to ensure attachment to substrate.

8. After 4 days in culture or longer, cells are ready for your use.

9. One-half medium should be exchanged once or twice a week with equilibrated medium.

10. Measure viability at 4 to 5 DIV. Exchange medium with warm NbAtiv4 or Hibernate E/B27/gln. Count live, phase-bright cells with processes. Small, bright cells are dead or apoptosing.

Alternatively a live-dead fluorescence assay can be conducted as follows:
Viability assay: viability=green cells per unit area/(total cells plated per unit area) or survival (green cells/(green + red cells)

a. Rinse 2 times with PBS or HBSS, 0.4 ml/2 cm^2 of substrate

b. From an acetone stock of 15 mg/ml fluorescein diacetate (Sigma #F7378), add 15 µl (1:100 dilution of the stock into 1.5 ml HBSS). From an aqueous stock of 4.6 mg/ml propidium iodide (Sigma #P4170), add 15 µl of the stock into the same 1.5 ml HBSS (1:100 dilution). Add 40 ul of that dilution to each well with 0.4 ml HBSS (further 1:10 dilution).

c. After about 1 min., count using Nikon B1A, G1B filters or other blue excitation appropriate for fluorescein fluorescence. Green cells are live. Small red nuclear stain indicates a dead cell.

d. If desired, fix and stain with 0.25% Coomassie blue R in ethanol/HAc/ water (45/10/45), 1 min., rinse with 10% HAc, aspirate and dry.