Mouse Neural Progenitor Culture (Neurosphere Assay)

In vitro culture of neural stem/progenitor cells from embryonic mouse brain

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Overview

The neurosphere assay is a method for culturing neural stem and progenitor cells (NSPCs) from embryonic or postnatal mouse brain. Cells are grown in suspension as free-floating aggregates (neurospheres) in the presence of EGF and FGF growth factors.

Workflow Summary:

- **Step 1:** Dissect telencephalic vesicles from E13-E15 embryos
- **Step 2:** Chemical dissociation (10 min at 37°C)
- **Step 3:** Trituration to single cells
- **Step 4:** Wash and resuspend in complete stem cell medium
- **Step 5:** Culture in ultra-low attachment dishes
- **Duration:** Neurospheres form in 48-72 hours
- **Passaging:** Dissociate and replate every 5-7 days

Applications

- Neural stem cell self-renewal studies
- Differentiation assays (neurons, astrocytes, oligodendrocytes)
- Drug screening on neural progenitors
- Developmental neurobiology
- Brain tumor stem cell studies
- Neurogenesis research

Key Features

- Maintains stem cell characteristics
- Self-renewing and multipotent
- Can be passaged for weeks
- Express Nestin, Sox2, GFAP markers
- Serum-free, defined medium
- Rapid proliferation (48-72h doubling)

Embryonic Age Considerations:

- **E13-E15:** Optimal for neurosphere formation, high yield
- **E16-E18:** Still viable but lower neurosphere-forming capacity
- **Postnatal (P0-P7):** Can be used, but neurosphere formation decreases with age
- **Adult SVZ/SGZ:** Requires specialized protocols, lower yield

Safety & Animal Care

- Ensure proper IACUC approval for all animal procedures
- Timed pregnancies required (E13-E15 embryos)
- Follow institutional guidelines for pregnant animal handling
- Work in biosafety cabinet for sterile technique
- Wear appropriate PPE: lab coat, gloves, eye protection
- Dispose of animal tissues according to institutional policy