

**Axon Outgrowth Assay by Explant Culture**

**Materials**

PBS ( 1X, PH 7.2 )	GIBCO #	20012-027
Leibovitz's L-15 medium	GIBCO #	21083-027
Matrigel™ basement membrane matrix	BD Biosciences #	354234
DMEM/F12 medium	GIBCO #	11320-033
Penicillin-streptomycin	GIBCO #	15140-122

**Procedure**

1. Before experiment, sterilize the dissection tools by soaking in 70% alcohol for about half an hour.
2. Suffocate the timed pregnant rat by CO<sub>2</sub> ( dry ice ) in a closed box, and then swab its abdomen with 70% alcohol and open it to expose the pups.
3. Remove all pups from the uterine horn, and then transfer them to a dish containing cold PBS buffer.
4. For cortical explants,
  - a) Excise the brains from the skull of E14-15 embryos, immediately dissect out the telencephalic vesicles, and then remove all visible pia in Leibovitz's L-15 medium under stereo-microscope ( Olympus SZX12)
  - b) Dissect out the dorsolateral cortex and cut it into approximately 400 X 400 μm pieces that comprise the entire thickness of the cortical wall by using a thin tungsten needle ( gift of Dr. Yi Rao )

For spinal cord explants,

- a) Dissect out the spinal cord from the E11-13 embryos in Leibovitz's L-15 medium, and strip away the meningeal covering.

- b) Cut the lumbar enlargement into 1000  $\mu\text{m}$  slices and separate into lateral halves with a thin tungsten needle.
  - c) Dissect out the dorsal portion ( upper third or half of the transverse section of the slices ).
5. After dissection, transfer the explant into a drop of approximately 20  $\mu\text{l}$  matrigel (The matrigel is commercially available and ready-to-use. It should be placed on ice always, otherwise it would polymerize at r.t.. Store the matrigel at  $-80\text{ C}^\circ$  ), place it in an appropriate orientation ( cortical explant oriented with the ventricular side up; dorsal spinal cord explant oriented with the transverse side up), and then cover it with another drop of 20  $\mu\text{l}$  matrigel.
  6. After gel polymerization, cover the gels with DMEM/F12 supplemented with 10% heat-inactivated fetal calf serum, and 20 U/ml penicillin/streptomycin, and then incubate the explants in a 5%  $\text{CO}_2$  atmosphere.
  7. Analyze the axon outgrowth after 16 hours in culture.