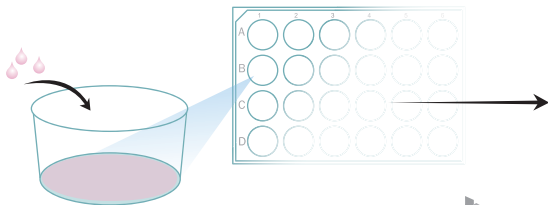


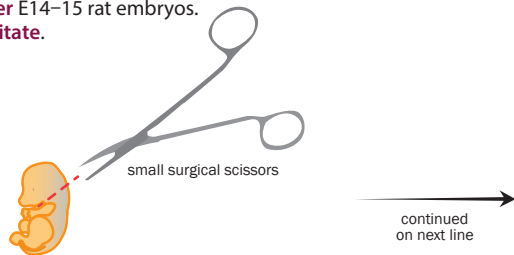
Day 1

Prepare cell culture plates by coating with Poly-D-Lysine.



Day 2

Recover E14-15 rat embryos.
Decapitate.



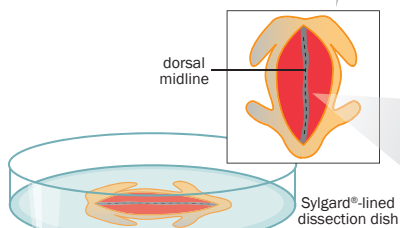
Place rat embryos dorsal side up in dissection dish. Expose dorsal spinal cord by removing skin and tissue. Open the spinal cord by cutting along the dorsal midline.

Vannas-Tübingen spring scissors

Continue to remove tissue to expose the dorsal root ganglia (DRGs). Separate DRGs from both sides of the spinal cord.

Remove isolated spinal cords. Trim off the dorsal column and cut into small pieces.

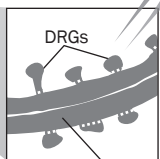
Transfer dissected spinal cord tissue. Centrifuge. Decant supernatant.



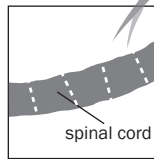
dorsal midline

Sylgard[®]-lined dissection dish

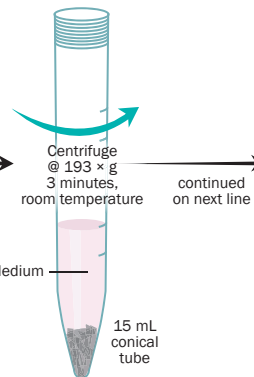
Dumont #5 forceps



spinal cord



spinal cord



Centrifuge @ 193 x g 3 minutes, room temperature

L15 Medium

15 mL conical tube

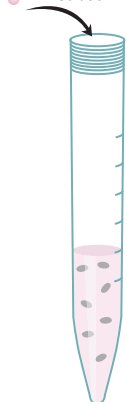
Digest the spinal cord tissue.

Stop the tissue digestion. Centrifuge. Decant supernatant.

Resuspend the spinal cord tissue. Triturate.

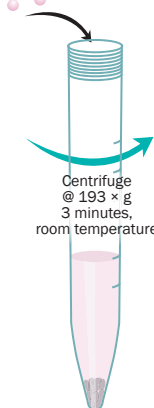
Divide the homogenized solution among 6 tubes containing the 9% OptiPrep[™] solution. Centrifuge.

3 mL 1:1 Trypzean[™]:DPBS Solution



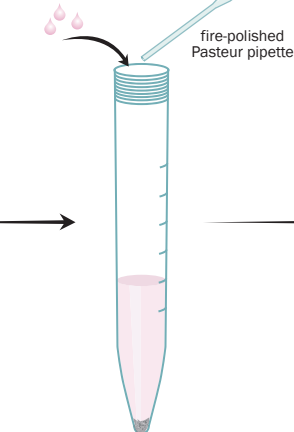
Incubate 15 minutes in a 37 °C water bath, gently agitate tissue.

3 mL FBS



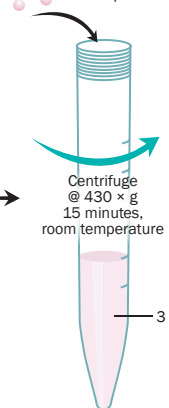
Centrifuge @ 193 x g 3 minutes, room temperature

6 mL L15 Medium



fire-polished Pasteur pipette

cell suspension



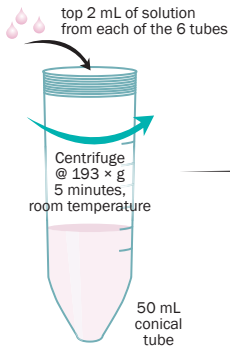
Centrifuge @ 430 x g 15 minutes, room temperature

x 6

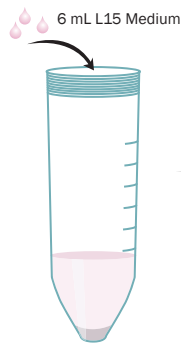
3 mL OptiPrep[™] Solution

continued on top of next page

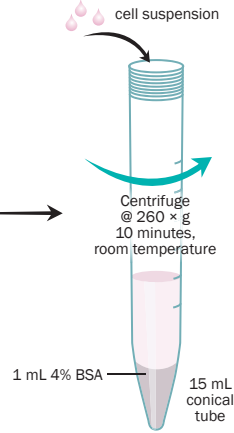
Transfer the top 2 mL of solution from each tube to one 50 mL conical tube. **Centrifuge**. **Decant** supernatant.



Resuspend the spinal motor neurons.



Transfer the cell suspension to a new 15 mL conical tube, layering it on top of the 4% BSA solution. **Centrifuge**. **Decant** supernatant.



Resuspend the spinal motor neurons in 250–500 μL of culture medium. **Count** cells.

Seed neurons onto Poly-D-Lysine-coated cell culture plates.

Add culture media to each well of the plate. **Culture** spinal motor neurons for desired amount of time. **Exchange** media every 3–4 days.

