Day 1

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

**Incubate** 15 minutes in a 37 ºC water bath, gently agitate tissue.

**3 mL FBS cell suspension**

**3 mL OptiPrep™ Solution**

**3 mL 1:1 Trypzean™:DPBS Solution**

**6 mL L15 Medium**

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**Day 2**

**Recover** E14–15 rat embryos. **Decapitate.**

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**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.

**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.**

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**Sylgard®-lined dissection dish**

**Vannas-Tübingen spring scissors**

**Dumont #5 forceps**

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**Fire-polished Pasteur pipette**

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**Centrifuge @ 193 × g 3 minutes, room temperature**

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**Centrifuge @ 430 × g 15 minutes, room temperature**

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

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

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

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

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.

Incubate 2 hours in a 37 ºC, 5% CO₂ humidified incubator.

Cell suspension

100 µL culture media

900 µL culture media