# DRG Cell Culture Protocol

**Check-list:**

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|---|---|---|---|
| | DMEM, high glucose, GlutaMAX™ | | 24-well plates |
| | Pen/Strep | | Glass coverslips |
| | FBS | | Glass Pasteur pipettes of various calibers + rubber pipettor |
| | Collagenase | | EtOH 70% |
| | NGF | | 15 mL Falcon tubes |
| | B27 | | 50 mL Falcon tubes |
| | BSA | | Sterile tips + pipettes |
| | Sterile 1x PBS | | Syringe + filter for BSA sterilization |
| | Laminin | | Cell Counter slides |
| | Poly-L-Lysine (PLL) | | |
| | PFA 4% | | |
| | Trypan Blue | | |

**Preparation before DRG collection**

1. Prepare the DRG collection medium: **DMEM: GlutaMAX + 1% Pen/Strep + 10% FBS**.

3. Distribute 2/3 mL of medium in 15 mL falcon tubes.

**Tissue digestion, dissociation and cell plating**

1. Collect DRG neurons from one animal and place them in the falcon tubes with 2/3 mL medium.

2. In the flow-cabinet, replace media with fresh one supplemented with **0,125% collagenase-IV-S** (**200 µL in 1.8 mL of medium**).

3. Incubate at 37$^{o}$ C for 1h30m (max 2h). Mix every 30/45 minutes.

4. Discard part of the supernatant, leaving a bit of collagenase for the following washing and dissociation steps.

5. Wash nodules 3x with 3 mL **DMEM: GlutaMAX + 1% Pen/Strep** (can contain FBS, if there is any medium left from previous steps), each time spinning the ganglia at 1000 rpm for 20 seconds (adjust to rotor radius!). Remove supernatant and replace with fresh medium after each spin.

6. Add 2 mL of **DMEM: GlutaMAX + 1% Pen/Strep** and dissociate the ganglia using at least three diameters of modified Pasteur pipettes (with decreasing tip diameter, using Bunsen burner flame). Stop when no visible clumps are present.

7. **OPTIONAL** - Put the 2 mL single cell solution in a **15% BSA cushion**. (300 mg of BSA in 2 mL of **DMEM: GlutaMAX + 1% Pen/Strep** in 15mL falcon tube – filter with 0.22 µm filter to sterilize). Pipette the cell solution carefully on top of the BSA cushion.

8. Spin at 6000 rpm for 6 minutes, RT. Remove the supernatant by starting with the interphase (if BSA cushion was used) and remove the maximum possible.

9. Resuspend the cell pellet in **1 mL** of **DMEM: GlutaMAX + 1% Pen/Strep (10 µL) + 20 µL B27 + 0.5 µL NGF [50 ng/mL].**

10. Count the cells using a Cell Counter (10 µL of cells + 10 µL of trypan blue).

11. Dilute as needed and plate the cells using 500 µl of culture medium (5000 to 6.500 cells p/well). Mix plate in "8" or "T" shape.

12. Place in 37°C, 5% $CO_2$ chamber for 24h.

## Coverslip coating

1. Place the coverslips in a 24 well plate.

2. Add 500 µL **EtOH 70%**, mix plate in "8" or "T" shape and then remove it.

3. Wash 3x with 1x sterile-PBS.

4. Add 500 µL of Poly-L-Lysine (**PLL**) (stock 10 mg/mL) solution in PBS 1x at 20 µg/mL in PBS (**1µL of PLL/well**). Incubate **1h** at 37$^{o}$ C, then remove it.

5. Wash 3x with **1x sterile-PBS** and air dry for **45 min** without the plate cover in the flow cabinet.

6. Add 500 µL of **laminin** (stock 1 mg/mL) solution in PBS 1x at 5µg/mL (**2.5 µL of laminin/well**). Incubate for **30 min** at **37$^{o}$ C** and then remove it.

7. Wash 3x with **1x sterile-PBS** and let the last wash sit in the wells until plating the cells. Don't let the coverslips/wells dry.

## Immunofluorescence protocol for adherent cells

Cell fixation:

1. Remove the culture medium.
2. Wash the cells gently with **1x PBS** and remove it.
2. Add 500 µL of **4% PFA** and incubate at RT for 15 minutes.
3. **OPTIONAL** - Wash 3x with **1x PBST** and store in 500 µL PBS at 4$^{o}$C until further use, if necessary.

ICC protocol:

1. Wash 3x with **1x PBST**.
2. Block with **5%** Natural Horse Serum (**NHS**) in PBST for 1h at RT.
3. Add 500 µL of primary antibody: **βIII-Tubulin** 1:1000 in 2% PBST. Incubate ON at 4$^{o}$C with agitation.
4. Wash 3x with **1x PBST** (5 minutes each).
5. Incubate with **secondary fluorescent antibody** 1:1000 for 1h at RT, in **2% PBST**, with agitation.
6. Wash 3x with **1x PBST**.
7. Wash 1-2x with **1x PBS**.
8. Incubate with **DAPI** 1:10 for 10 min.
9. Wash 1-2x with **1x PBS**.
10. Mount slides with ProLong Gold Antifade Mountant.