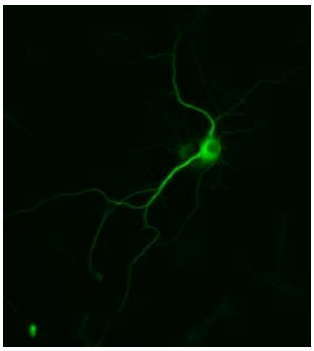
REF: P20202

TTFLUOR™ DRGs

GREEN FLUORESCENT DORSAL ROOT GANGLION CELLS



Product Name:	TTFLUOR DRGs
Catalog Number:	P20202
Cell Type:	Primary Rat Dorsal Root Ganglion Cells
Fluorescent Protein:	turboGFP (Evrogen)
Format:	> 5 x 10^5 cells in Cryopreserved vials
Storage:	Liquid Nitrogen

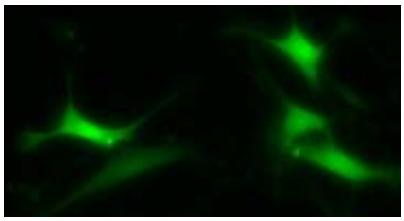
TTFFLUOR DRGs are Green fluorescent primary DRG cells, which has been developed through transient transfection with tGFP expression vector expressing the green fluorescent protein gene sequences as free cytoplasmatic protein.

About primary DRGs

Dorsal root ganglion is a group of sensory nerve cell bodies. They pass sensory information to neurons in the spinal cord so it can be analyzed by the brain. In anatomy and neurology, the dorsal root ganglion (or spinal ganglion) is a nodule on a dorsal root that contains cell bodies of neurons in afferent spinal nerves. Cultured adult rat dorsal root ganglion (DRG) neurons can be used to study depolarization-induced Ca2+ mobilization and the effects of intracellular Ca2+ depletion on neurite outgrowth. DRGs are very useful to evaluate the neuroprotective & neurotoxicity effects of new agents.

Recommended Medium

- Neurobasal (Invitrogen ref: 21103-049)
- B27 (Invitrogen ref: 17504-044)



Within 2-3 days in culture, the fluorescent DRG neurons display extensive neurite outgrowth. On day 10 approximately they show the same size and neurochemical type distribution as freshly dissociated rat DRG neurons. This is consistent with the generally accepted view that after 9 days in culture, DRG neurons are physiologically mature.

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INSTRUCTIONS FOR CULTURING CELLS

IMPORTANT: Cryopreserved cells are very delicate. Thaw the vial in a 37 ºC waterbath and return them to culture as quickly as possible with minimal handling!

Unpacking:

1. For cryopreserved cells: If there is dry ice in the package and you are not going to culture cells right way, place cryovial(s) immediately into liquid nitrogen. If there is no dry ice left in the package, thaw and culture the cells immediately.

2. For proliferating cells: Spray the culture vessel (flask, plate or slide) with 70% ethanol for disinfection. Transfer the cells into 37ºC, 5% CO_2 incubator and allow equilibrating for 2 hours. After cells have equilibrated, remove shipping medium from the culture vessel and replace with fresh medium.

Set up culture after receiving the order:

1. Coat culture vessel with laminin or poly-L-lysine. *Note: It is important that neurons are plated in laminin or poly-L-lysine coated culture vessels that promote cell attachment and neurites outgrowth (poly-L-lysine coating: coat flask or plate with poly-L-lysine at 2 µg/ml concentration for one hour and wash the flask or plate with sterile water three times).*

2. Medium preparation: Decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with the medium to recover the entire volume.

3. Set up culture: Prepare one T-45 flask for each cryovial. Add the appropriate amount of medium to the vessel (recommend for 10 ml/T-45 flask) and allow the flask to equilibrate in 37ºC, 5% CO_2 incubator for at least 30 min.

4. Thawing of cells: Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, and transfer it to a sterile field. Rinse the vial with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers.

5. Using 1 ml eppendorf pipette gently resuspend the cells in the vial and transfer them to equilibrated culture vessels (a T-45 flask). A high seeding density (>10,000/cm^2) is recommended. *Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture.*

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange. Return the culture vessels to the incubator.

7. Change the medium 4 - 6 hours after plating to remove the residual DMSO and unattached cells, then every other day thereafter. A health culture will display normal neuron morphology (unipolar or dipolar processes with small and round phase-bright cell body in early days of culture).

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Caution: *Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].*

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods.* 11(4).

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