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# Product Information

**Rat Cortical Neurons (RCN)**

| | | | |
|---|---|---|---|
| Catalog Number | 10RA-032 | Cell Number | 2.0 million cells/vial |
| Species | *Rattus norvegicus* | Storage Temperature | Liquid nitrogen |

## Product Description

Cortical neurons make up a large portion of the brain and consist of both glutamateric and GABA-ergic neurons [1]. They play an important role in higher level thought processes and are necessary to organize input from subcortical structures. Like all neurons, these cells transport Na+ that work to propagate action potentials from one cell to the next, which is the basis of a working neural pathway. These cells can be used to study a variety of cortical related abnormalities including Huntington's Disease, Alzheimer's Disease, psychiatric disorders, and learning disorders [2, 3].

iXCells Biotechnologies provides high quality Rat Cortical Neurons (RCN), which are isolated from the brains of E16 embryonic rats. These cells are cryopreserved at P0, with ≥ 2 million cells in each vial. They are negative for mycoplasma, bacteria, yeast, and fungi. RCNs can be plated using Rat Cortical Neuron Recovery Medium (cat# MD-0107A) and maintained in Rat Cortical Neuron Maintenance Medium (Cat # MD-0107B) under the condition suggested by iXCells Biotechnologies.

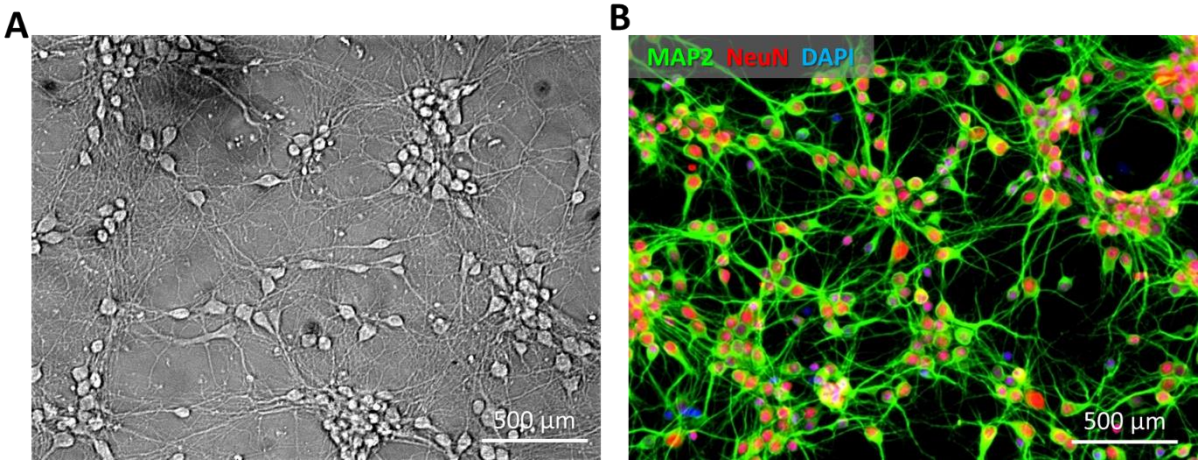


**Figure 1.** Rat Cortical Neuron (Cat# 10RA-032) were recovered and cultured for 5 days. (A) Phase contrast image. (B) Immunofluorescence staining were performed using antibodies against the neuronal markers MAP2 (green) and NeuN (red).

## Product Details

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|---|---|
| **Tissue** | D16 embryonic rat brain tissue |
| **Package Size** | 2.0 million cells/vial |
| **Passage Number** | P0 |
| **Shipped** | Cryopreserved |
| **Storage** | Liquid nitrogen |
| **Growth Properties** | Adherent |
| **Media** | Rat Cortical Neuron Recovery Medium (Cat # MD-0107A)<br>Rat Cortical Neuron Maintenance Medium (Cat# MD-0107B) |

## Protocols

### Poly-L-Lysine coating protocol

1. Add 100 µL/cm$^2$ 0.01% PLL (Sigma-Aldrich, Cat# P4707-50ml) in the desired culture vessels.
2. Rock gently to ensure even coating of the culture surface and incubate at room temperature from 4 hours to overnight.
3. After incubation, remove solution by aspiration and thoroughly rinse surface with sterile tissue culture grade water. Perform 6 washes of at least 20 minutes each with sterile tissue culture grade water.
4. Allow to dry at least 2 hours before introducing cells and medium.

### Thawing of Frozen Cells

1. Equilibrate 20 mL **Rat Cortical Neuron Recovery Medium** (Cat# MD-0107A) for 1 hour in 37°C, 5% CO2 humidified incubator prior to unthawing cells.
2. Upon receipt of the frozen Rat Cortical Neurons (RCN), immediately place into liquid nitrogen storage or thaw the cells and initiate the culture immediately in order to retain the highest cell viability.
3. To thaw the cells, put the vial in 37°C water bath with gentle agitation until the contents completely thaw. Keep the cap out of water to minimize the risk of contamination.
4. Resuspend the cells by gently pipetting the cells 2 times with a 1 mL pre-wetted aerosol tip set at 950 µL. Be careful not to pipette too vigorously as to cause foaming or bubbles.
5. Transfer the cell suspension from the vial into a 50 mL tube. Dropwise add 19 mL equilibrated Rat Cortical Neuron Recovery Medium (Cat# MD-0107A) while swirling the tube to mix. Rinse the cryovial to recover all the content and collect the medium to the tube.
6. Gently mix the cell suspension in the 50 mL tube by trituration in a 10 mL pipette Count cells and aliquot into each well of the desired size plate. A seeding density of 52,000 cells/cm$^2$ is recommended. Rock gently to evenly distribute the cells.
7. Place the plate in a 37°C, 5% CO2 humidified incubator; for best results, do not disturb the culture for 24 hours after initial seeding.

8. Change to fresh pre-equilibrate **Rat Cortical Neuron Maintenance Medium** (Cat# MD-0107B) after 24 hours.
9. Change media with Rat Cortical Neuron Maintenance Medium (Cat# MD-0107B) every 2-3 days; Make sure to remove media with pipette in tilted plate (do not aspirate media off as neurons can come off the plate easy), and add media back in with pipette targeted towards the side of the well, as to disturb the cells as little as possible.
10. If not all cells attach to the plate and removal of dead cells is necessary, while doing media change, remove media and then add media dropwise to the center of the well, remove this media and then replace with media as usual (targeted against side of well so as to disturb cells as little as possible).
11. The cells will be ready for use in an assay after 4 - 5 days in culture.

**Safety Precaution:** *it is highly recommended that protective gloves and clothing should be used when handling frozen vials.*

**Note:** *It is not recommended to passage the RCN because the cells will most likely not survive the re-plating process.*

# References


[1] Johnson M, Walsh C. (2017) " Cerebral Cortical Neuron Diversity and Development at Single-Cell Resolution " Curr Opin Neurobiol. 42: 9-16.

[2] Lim L, Mi D, Llorca A, Marin O. (2018) " Development and functional diversification of cortical interneurons." Neuron. 100(2): 294-313.

[3] Molnár Z, Clowry G J, Šestan N, Alzu'bi A, Bakken T, Hevner R F, et al. (2019) " New insights into the development of the human cerebral cortex." J. Anat. 235: 432-451.


## Disclaimers