NEUROMICS

# P8 Primary Rat Cerebellar Cells

**Catalog Number:** PC35103

**Components:**
- **Approximately 1 x $10^7$ cells (2 mls)**
  P8 Sprague/Dawley or Fischer 344 Rat Cerebellar Cells
- **12 mls Culture Media**
  Neurobasal/B27/0.5 mM glutamine culture medium

**Description:** Primary Rat Cerebellar Cells are live neurons isolated from micro-surgically dissected regions of post-natal Day 8 Sprague/Dawley or Fischer 344 rat brain. These cells are prepared fresh each week and shipped in a nutrient rich medium that keeps the cells alive for up to 14 days under refrigeration.

**Shipping/Storage:** Primary Rat Cerebellar Cells are shipped refrigerated. Cells are stable for up to 2 weeks when stored at 4-8ºC. It is recommended to plate the cells as soon as possible after receiving cells according to Application Notes outlined below.

## Application Notes

### Substrate Preparation

1. Prepare culture plate by coating with poly-D-lysine (0.15 ml/cm$^2$, 50 µg/ml, 135 kD) 1-20 hr., and rinse one time with 18 Mohm deionized water, and let dry.

### Preparation of Isolated Neurons

1. After receiving the cells, let them settle at 4°C for 2 hours, OR spin down at 1,100 rpm (200xg) for 1 min.
2. Transfer 1 ml of medium from the cells tube into a 50ml screw cap sterile tube; be careful not to disturb or remove cells from the original cells tube.
3. Using a 1 ml pipettor with a sterile blue plastic tip, or a silanized 9-inch Pasteur pipette with the tip barely fire polished (preferable), suck the cells with the medium into the pipette and immediately dispense the contents back into the same container. Take care not to create bubbles. Repeat this tituration step about 10 times or until most all the cells are dispersed.
4. Transfer the dispersed cells into the 50ml tube that contains the 1ml of media from Step 2, and gently mix the cells by swirling.
5. Spin the cells at 1,100 rpm (200xg) for 1 minute. Discard the supernatant while being careful not to remove any of the cells from the cell pellet.
6. Flick the tube a few times to loosen the cell pellet. Resuspend pellet in 1 ml of the provided B27/Neurobasal/0.5 mM glutamine/25 µM glutamate medium. Resuspend cells by gently pipetting up and down.
7. Aliquot 20 µl and mix with 20 µl of 0.4% trypan blue.
8. Count cells with a hemocytometer.
9. Further dilute the cells with B27/Neurobasal/0.5 mM glutamine to the desired plating density. We recommend 32 x $10^3$ cells/2 cm$^2$ of substrate in 0.4 ml/2 cm$^2$ substrate.
10. Incubate the cells at 37ºC with 5% $CO_2$ and/or 9% or 20% oxygen.
11. After 4 days or longer, neurons are well differentiated. If further culture is desired, change half of medium with fresh, warm B27/Neurobasal/0.5 mM glutamine (you will need to purchase additional medium for longer culture times). Change half the medium every 3-4 days.

### Viability Assay

1. Rinse cells twice with PBS.
2. From an acetone stock of 15 mg/ml fluorescein diacetate (Sigma), add 15 µl (1:100 dilution of the stock) into 1.5 ml HBSS. From an aqueous stock of 4.6 ml/ml propidium iodide, add 15 µl of the stock into the same 1.5 ml HBSS (1:100 dilution). Add 40 µl of that dilution to each well with 0.4 ml HBSS (further 1:100 dilution).
3. After approximately 1 minute, count using Nikon B1A filter or other blue excitation appropriate for fluorescein fluorescence. Green cells are alive. Small red nuclear stain indicates a dead cell.
4. If desired, fix and stain with 0.25% Coomassie blue R in ethanol/HAc/water (45/10/45), 1 min., rinse with 10% HAc, aspirate and dry.



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