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NeuroFreeze Kit

Catalog Number M036041

For the preservation of primary neurons from rat / mouse hippocampi and cortices.

For research use only.
This product insert must be read in its entirety before use.

SUMMARY AND EXPLANATION

The NeuroFreeze kit can be used to freeze primary neurons from E18/19 rat and P0/P1mouse hippocampi and cortices. Neurons frozen in this media maintain high levels of viability (75-90%) upon thawing. These neurons show all the expected characteristics of primary neurons, including appropriate morphology and expression of neuronal and synaptic markers. The availability of this media will enable investigators to freeze primary neurons from embryonic rats (E18) or P0/P1 wild type or genetically engineered mice.

The NeuroFreeze kit is available in two sizes. The kit comes with four components and can be utilized to freeze and thaw neurons using the following protocols.

KIT COMPONENTS

Catalog number: M036041-A

NeuroFreeze Kit

Size: 10mL Kit
Preservation ≈ 10 vials of cells

Component A - 1 vial, 10mL

Component B - 1 vial, 1mL

Component C - 1 vial, 100µL

Component D - 1 vial, 30mL

Catalog number: M036041-B

NeuroFreeze Kit

Size: 30mL Kit
Preservation ≈ 30 vials of cells

Component A - 1 vial, 30mL

Component B - 2 vial, 1.5mL

Component C - 1 vial, 300µL

Component D - 2 vials, 2 x 45mL

STORAGE

<table>
<tr><td>Unopened kit</td><td colspan="2">Component C needs to be stored at -20 ºC. All other Component are stored at 2 - 8 ºC. Do not use past the kit expiration date.</td></tr>
<tr><td rowspan="4">Opened/Reconstituted Reagents</td><td>Component A</td><td rowspan="2">Store at 2 - 8 ºC</td></tr>
<tr><td>Component D</td></tr>
<tr><td>Component B</td><td>Store at 2 - 8 ºC Keep protected from light. Thaw at RT.</td></tr>
<tr><td>Component C</td><td>Store at -20 ºC Keep protected from light. Thaw at RT. Avoid repeated freeze-thaw cycles. It is recommended to make aliquots upon receiving the kit. Discard after 4 freeze-thaw cycles.</td></tr>
</table>

PRECAUTIONS

- Each time a new pipette tip is used, aspirate a sample or reagent and dispense it back into the same vessel.
- For research laboratory use only. Not for human diagnostic use.
- Do not pipette liquids by mouth.
- Do not use kit reagents beyond the expiration date.
- Do not eat, drink or smoke in area in which kit reagents are handled.
- Avoid splashing.

PROTOCOL

Freezing Protocol

1. Obtain dissociated primary neurons from rat/mouse hippocampus/cortex as described (Beaudoin et al). Re-suspend dissociated cells in minimal volume of plating media.
2. Estimate cell viability on a Hemocytometer.
3. Do not exceed a maximum of 4 million cells/vial for freezing. It is recommended to freeze 1 to 4 million cells per vial.
4. Re-suspend cells to be frozen in 900µl of Component A.
5. Add 10% (100µl for 1mL) of Component B to the vial.
6. Add 1% (10µl for 1mL) of Component C to the vial.
7. Gently mix and place the vial in isopropanol bath in -80°C freezer.
8. Next day, place vial(s) in liquid nitrogen.

Note: Steps 1-6 should be carried out at RT.

Thawing Protocol

1. Follow your laboratory's protocol for coating substrate.
2. Add pre-maintenance media (Component D) to dishes/plates and incubate in 37°C incubator for a minimum of 2 hours (recommended 4 hours).
3. Bring a vial of cells from liquid nitrogen and quickly thaw in 37°C water bath (usually takes about 90 seconds) – it is critical that thawing is accomplished as fast as possible.
4. Estimate cell viability.
5. Seed cells onto the substrate **(See Plating Density Chart below).**
6. After cells have attached to the substrate (3-4hours), replace pre-maintenance media with your lab's neuron maintenance media (B-27 containing maintenance media is recommended).
7. Maintain neurons in B27 containing maintenance media at 37°C.

The plating density chart can be used to determine cell density and pre-maintenance media volume.

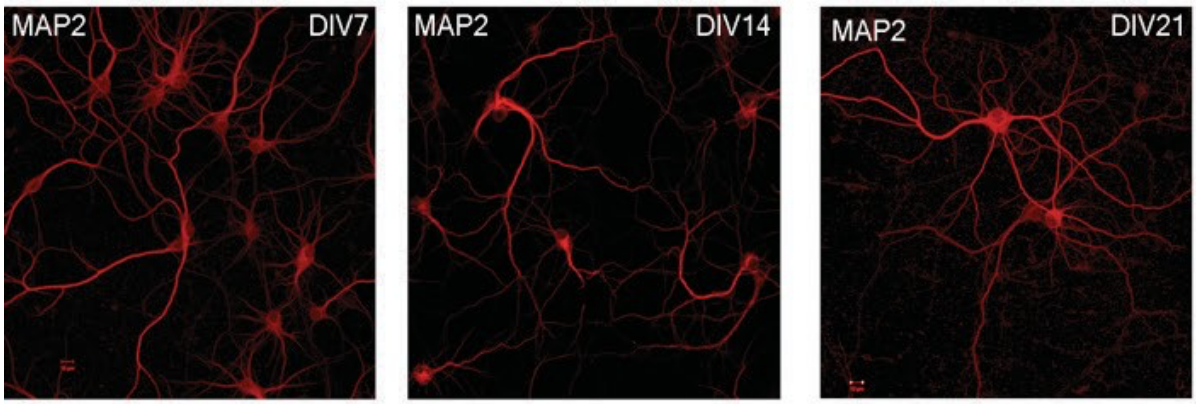
Plating Density Chart

Substrate (poly-lysine coated)	Cell Density*	Pre-maintenance Volume
100mM dish	2×10^6	7mL
60nM dish	1×10^6	4mL
12 wells plate	100,000	1mL
24 wells plate	50,000	0.5mL
96 wells plate	15,000	0.1mL

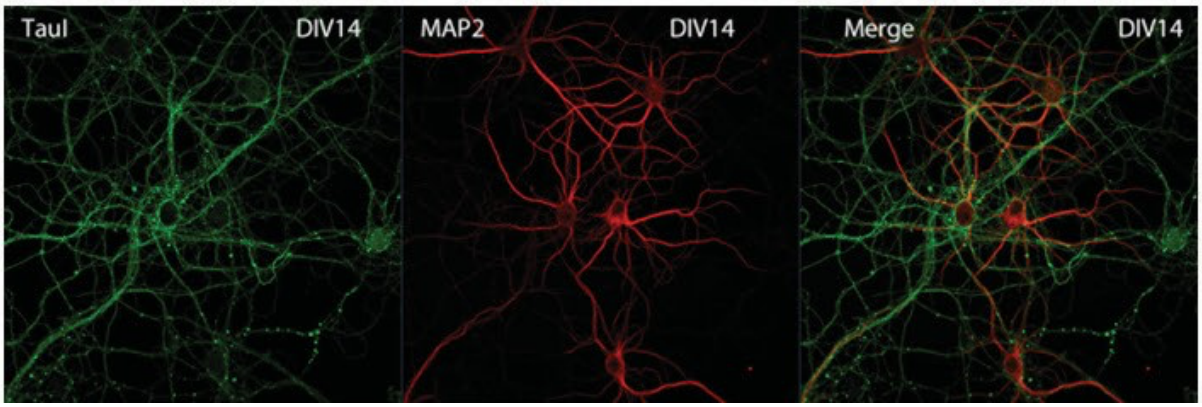
** These densities can be adjusted according to user requirements*

IMAGES AND CHARACTERIZATION

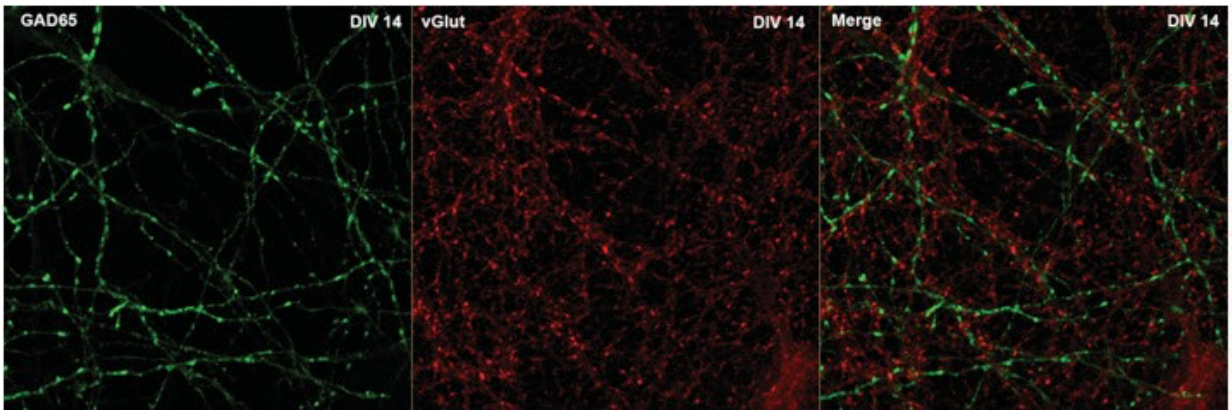
Rat Cortical Neurons at DIV 7, 14, and 21 stained with marker MAP2



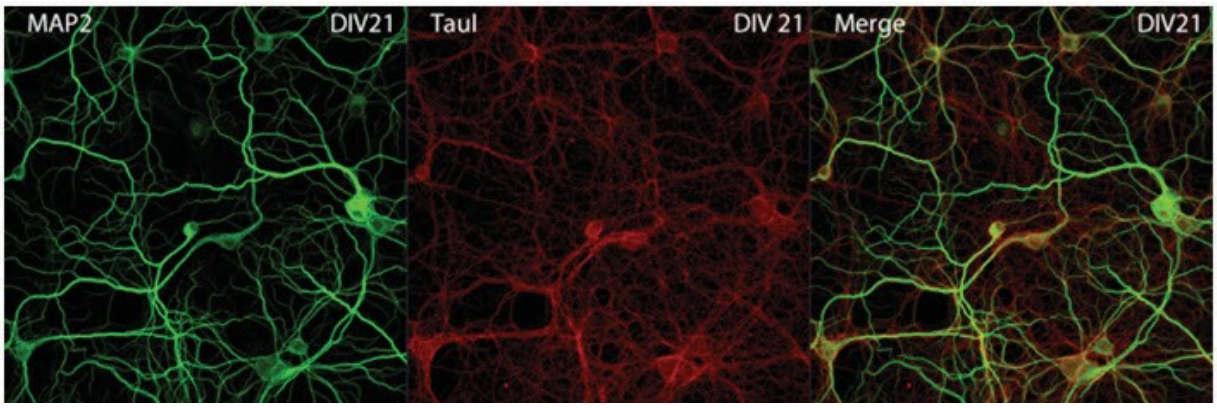
Rat Cortical Neurons at DIV 14 stained with markers TauI and MAP2



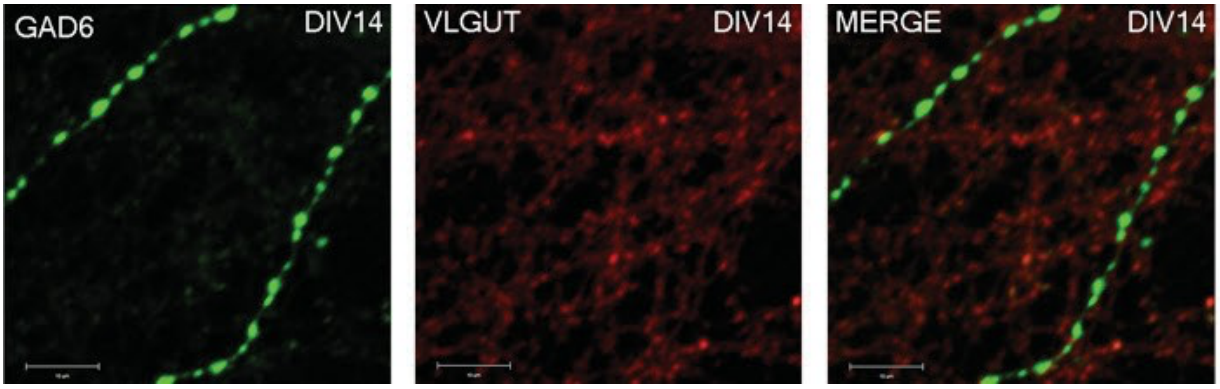
Rat Cortical Neurons at DIV 14 stained with markers GAD65 and VGlut



Mouse Cortical Neurons at DIV 21 stained with markers MAP2 and TauI



Mouse Cortical Neurons at DIV 14 stained with markers GAD65 and VGLUT



REFERENCES

1. Beaudoin GM 3rd, Lee SH, Singh D, Yuan Y, Ng YG, Reichardt LF, Arikkath J. Culturing pyramidal neurons from the early postnatal mouse hippocampus and cortex. Nature Protocols, 7(9):1741-1754 (2012).

NOTES

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