MTI-GlobalStem

Application Note

Primary rat and mouse neuronal cultures are widely used by researchers studying the basic physiological properties of neurons and the mechanisms of cognitive and neurological disorders that impact people.

Some scientists work with neurons in small scale experiments while others are developing large scale high throughput screening assays requiring enormous numbers of primary neuronal cells.

In most cases, laboratories dissect rats and plate fresh neurons prior to every experiment.

This may appear to be the best way to ensure quality neurons for important experiments, but is this the only way? Is there an alternative?

Cryopreserved Neurons – A Better Alternative?

Protocols for isolation of neurons can be found all over the internet, recommending a variety of reagents and materials for use in culturing fresh primary neurons. Validating the materials, finding a protocol that works, and training personnel require a lot of time and money but are essential for reproducible cultures.

A typical timed-pregnant rat averages between 8-12 embryos. This provides a very good yield of cells for experimental purposes and if isolated correctly the viability can be greater than 50%.

In planning experiments, availability of timed-pregnant rats and consistency of gestation age are critical. If timed pregnancies vary in developmental age by as little as a day, the experimental cultures may vary in maturity and mixture of neuronal subtypes. This variation can be impossible to avoid if availability issues cause a researcher to switch rat strains or even suppliers.

In addition, microbiological contamination below the level of visual detection can greatly impact experimental results. When isolating neurons in-house, sterility and mycoplasma tests are frequently skipped due to cost and time constraints.

Bottom line, the serial preparation and cultivation of primary neurons is labor intensive requiring skilled personnel and long-term planning efforts.

There is a belief that frozen neurons will never be as good as fresh and some scientists are resistant to making changes to their experimental protocols but there are some benefits to using cryopreserved neurons.

Purchasing cryopreserved neurons provides more flexibility in the culture process eliminating the need for timed pregnant animals when a new experiment is planned.[1] With this, there's no need to pull important personnel away from other assays to perform dissections. With GlobalStem cryopreserved rodent neurons, a detailed protocol is provided with the cryopreserved neurons listing all materials necessary to ensure their survival.

CONTACT / ORDERING INFORMATION
MTI-GlobalStem • 200 Perry Parkway, Ste. 1. • Gaithersburg • Maryland • 20877
www.MTI-GlobalStem.com • info@globalstem.com • phone 301-545-0238 / 888-545-0238 • fax 301-424-1989

Lot to lot consistency is guaranteed due to high quality control standards. Mycoplasma and sterility testing are done for every lot.

Functionality of primary neurons after cryopreservation is always a question.[2] Data in (Fig. 1) conducted by researchers at Galenea, Corp. measured the pre-synaptic release of GlobalStem cryopreserved primary rat neurons compared with freshly prepared rat neurons. Both the fresh and the cryopreserved primary cells underwent stimulation and the synaptic release responses were shown to be equivalent (Fig. 1).

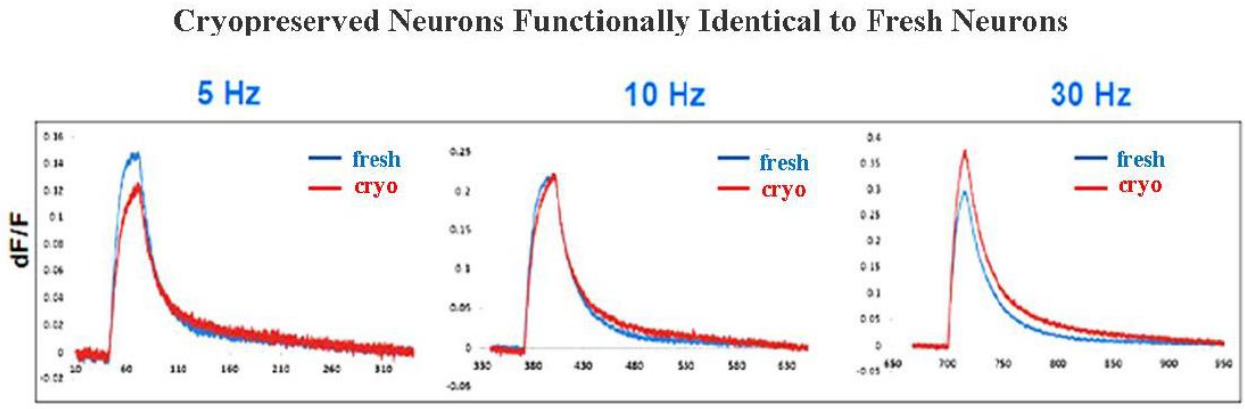

Figure 1. Evoked Synaptic Release Activity - Cryopreserved (red trace) and freshly isolated (blue trace) primary rat neurons were each plated at 80,000 cells per well and grown for 3 weeks. All the cells in the well were stimulated simultaneously. Above data shows GlobalStem cryopreserved primary rat neurons exhibit evoked synaptic release responses virtually identical to that of fresh isolated rat forebrain neurons. The fluorescent readout was a measure of presynaptic release. *Data courtesy of Pascal Laeng (Galenea)*

Another question often asked is can these cells be used for large-scale studies such as high throughput screening? The ability to cryopreserve large numbers of primary neurons while providing viabilities above 50% post thaw consistently is very important. Below, GlobalStem primary rat cortical neurons (Cat#: GSC-8220) and mouse cortical neurons (inquire) show a healthy morphology and maintain their ability to mature post-thaw when grown in NeuralQ™ Basal Medium (Cat#: GSM-9420) and GS21Neural Supplement (Cat#: GSM-3100) (Fig. 2).

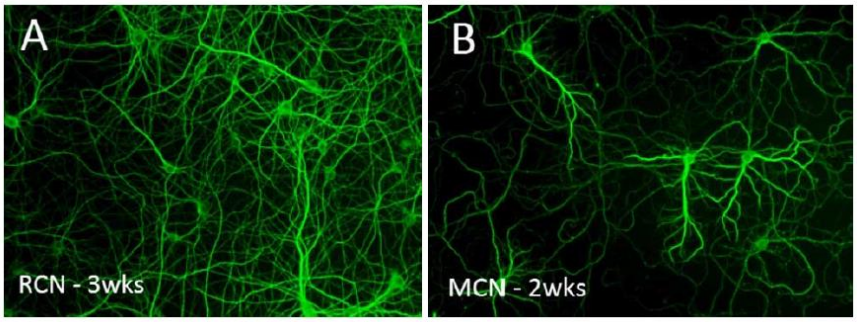

Figure 2. Healthy, mature primary cortical neurons post-thaw - Cryopreserved primary rat cortical neurons (Cat#: GSC-8220) (A) and mouse cortical neurons (B) were thawed and cultured for two and three weeks, respectively. MAP-2 (green) staining shows greater than 90% of the isolated cells are neurons.

CONTACT / ORDERING INFORMATION
MTI-GlobalStem • 200 Perry Parkway, Ste. 1. • Gaithersburg • Maryland • 20877
www.MTI-GlobalStem.com • info@globalstem.com • phone 301-545-0238 / 888-545-0238 • fax 301-424-1989

While isolating and using fresh neurons may seem like the right way to go initially, when considering the cost of ordering animals, supplies and hiring trained personnel, cryopreserved neurons is likely the better alternative. With MTI-GlobalStem's cryopreserved primary neurons the outcome is a high percent of viable, functional cells that maintain the ability to mature post-thaw. The user needs only to thaw and plate the cells, leaving them with more time to work on other lab responsibilities.

1. F. Otto et al. Journal of Neuroscience Methods 128, 1-2 (2003) 173-181.
2. Anders Lindgren. The European Life Science Journal for International Business 2 (2008) 72-73.

CONTACT / ORDERING INFORMATION
MTI-GlobalStem • 200 Perry Parkway, Ste. 1. • Gaithersburg • Maryland • 20877
www.MTI-GlobalStem.com • info@globalstem.com • phone 301-545-0238 / 888-545-0238 • fax 301-424-1989