BrightCell™ Photostable Media System

Catalog No. SCM143, SCM144, SCM145, SCM146, SCM147

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Not for use in diagnostic procedures.

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Introduction

Experiments with live cultured cells, including fluorescence microscopy, optogenetics, and fluorescence activated cell sorting (FACS) often entail high levels or prolonged exposure to blue light. However, cell culture media and supplements contain components that are converted to toxic free radicals by light. In particular, DMEM (Dulbecco's Modified Eagle Medium) and Neurobasal® medium, as well as cell culture supplements such as B-27®, SATO and NS21, can lead to significant perturbation of cellular behavior and marked increases in cell death. This phenomenon was recently highlight in the following nature publication:

Ragnhildur T. Káradóttir et. al. **Surpassing light-induced cell damage in vitro with novel cell culture media**. Nature Scientific Reports. Sci Rep. 2017 Apr 12;7(1):849.

The BrightCell™ photostable media system is cell culture media and supplements that have been reformulated with specific phototoxic components eliminated or replaced. The products allow prolonged exposure of cells to blue light while maintaining high levels of cell viability and functionality. Furthermore, BrightCell media products have very low levels of auto-fluorescence and photo-bleaching, dramatically improving the quality of data that can be obtained using fluorescent live cell imaging.

MEMO® supplemented with SOS® during exposure to prolonged periods of light results in significantly higher levels of cell viability. MEMO is used as a replacement for DMEM prior to and during exposure to light.

NEUMO® is a photostable medium which has been specifically developed for the culture of neuronal cells and can be used to replace Neurobasal® Media. Use of NEUMO supplemented with SOS during exposure to prolonged periods of light results in significantly higher levels of neural cell viability and fluorescence signal intensity.

SOS® is a photostable, serum-free, neuronal/stem cell supplement that maintains an essential level of proteins needed for cell culture with potent anti-oxidants. SOS has been designed to directly replace phototoxic serum-free supplements such as B-27®, NS21, SATO and N2. SOS should be used throughout the experiment and works well with standard DMEM/Neurobasal as well as with MEMO/NEUMO media.

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Materials Provided

1. BrightCell **MEMO®** Photostable Media, 100ml (Part No. SCM143): One (1) bottle containing 100ml of MEMO® Photostable Media (SCM143-1) Store at 2-8°C and One (1) bottle containing 1ml of Supplement A (SCM148) Store at -20°C.
2. BrightCell **MEMO®** Photostable Media, 500ml (Part No. SCM144): One (1) bottle containing 500ml of MEMO® Photostable Media (SCM144-1) Store at 2-8°C and One (1) bottle containing 5ml of Supplement A (SCM149) Store at -20°C.

3. BrightCell **NEUMO®** Photostable Media, 100ml (Part No. SCM145): One (1) bottle containing 100ml of NEUMO® Photostable Media (SCM145-1) Store at 2-8°C and One (1) bottle containing 1ml of Supplement A (SCM148) Store at -20°C.
4. BrightCell **NEUMO®** Photostable Media, 500ml (Part No. SCM146): One (1) bottle containing 500ml of NEUMO® Photostable Media (SCM146-1) Store at 2-8°C and One (1) bottle containing 5ml of Supplement A (SCM149) Store at -20°C.

5. BrightCell **SOS®** Neuronal Supplement (25X), 50ml (Part No. SCM147): One (1) bottle containing 50ml f SOS® Neuronal Supplement (25X) (SCM147-1) Store at -20°C and One (1) bottle containing 5ml of Supplement A (SCM149) Store at -20°C.

Storage and Stability

MEMO/NEUMO media should be stored at 2 to 8°C for up to 1 year. SOS supplement and Supplement A should be stored at -20°C for up to 1 year from manufacture. Avoid freeze-thaw cycles.

Materials Required But Not Supplied

BrightCell™ Photostable Media may require formulation optimization depending on the cell type used. Recommended medium supplementation for non-neuronal cells and neuronal cells are listed below and can be used as a starting guide:

Cell type	Component	Catalog Number	Stock Concentration	Amounts Required (100 ml)	Amounts Required (500 ml)
Neuronal Cells	NEUMO®	SCM145, SCM146	1X	100ml	500ml
	SOS®	SCM147	25X	4ml	20ml
	Human Recombinant Insulin	91077C (Sigma)	4mg/ml	375µL	1875µL
	Glutamine	A8185 (Sigma)	100X	1ml	5ml
	T3 (3,3'',5-Triiodo-L-thyronine)	T6397 (Sigma)	2mg/ml in 0.1M NaOH	25µl	125µl
	T4 (L-thyroxine)	T1775 (Sigma)	2mg/ml in 0.1M NaOH	25µl	125µl
Non-Neuronal Cells	MEMO®	SCM143, SCM144	1X	100ml	500ml
	SOS®	SCM147	25X	4ml	20ml
	Human Recombinant Insulin	91077C (Sigma)	4mg/ml	375µL	1875µL
	Supplement A	SCM148, SCM149	9.9%w/v	1ml	5ml

Table 1. Suggested media formulations to use on neuronal and non-neuronal cells with BrightCell Photostable Media.

Protocol

Some of the phototoxic components present in standard media (but removed from MEMO/NEUMO) contribute to cellular proliferation. Consequently, MEMO/NEUMO should only be used during the phase of the experiment in which cells are exposed to prolonged periods of light. SOS, in contrast, supports cellular viability and proliferation equal to alternative (phototoxic) supplements and should be used continuously during cell maintenance.

SOS® should be used to replace neuronal supplements, such as B-27®, if you are currently using these in your media. If you are presently using FBS, this can be harmful to cells under intensive light. Replacement of FBS with SOS® may help to support the growth of cells, especially when used in conjunction with cell line specific growth factors.

Media Preparation

1. Thaw SOS at 37°C. SOS is supplied as a 25 x concentrate and should be added to MEMO/NEUMO to a final concentration of 4%. Any liquid remaining should be aliquoted into working volumes and store at -20°C.

Note: Avoid freeze-thawing of SOS® supplement more than twice.

2. Add additional components in an aseptic manner according to Table 1 shown above.
3. Once supplemented, the complete media is stable for 2 weeks when stored at 4°C.

Cell Culture

1. Initially expand/maintain cells in the dark using Neurobasal supplemented with SOS.
2. The evening before exposure to prolonged periods of light, replace Neurobasal/SOS with pre-warmed MEMO/NEUMO and SOS. Cells will maintain viability for up to 3 days if required.

    a. Aliquot media to pre-heat, repetitive heating and cooling of bottles of media may cause precipitation.

3. Perform experiment requiring exposure to light.
4. Once exposure to light has been completed, remove the MEMO/NEUMO and SOS and replace with Neurobasal/SOS.

Data Analysis

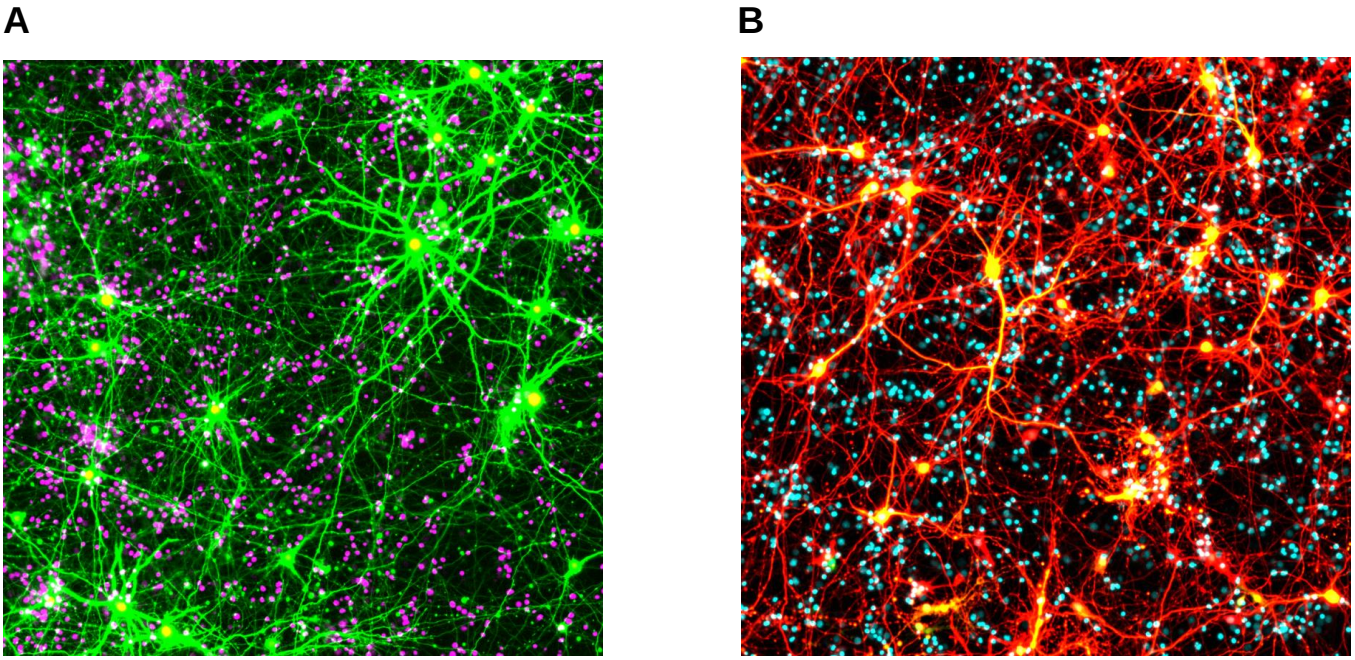

Figure 1. Live cell fluorescent imaging of neurons in BrightCell Media (NEUMO/SOS). A) Primary rodent cortical neurons expressing mApple (EGFP) and a mutant gene Matrin3 (YFP) involved in familial amyotrophic lateral sclerosis, counter-stained with a nuclear dye (purple). B) Live primary rodent cortical neurons expressing mApple (red) and a mutated gene C9orf72 (green) involved in familial amyotrophic lateral sclerosis and frontotemporal dementia, stained with a nuclear dye (cyan).

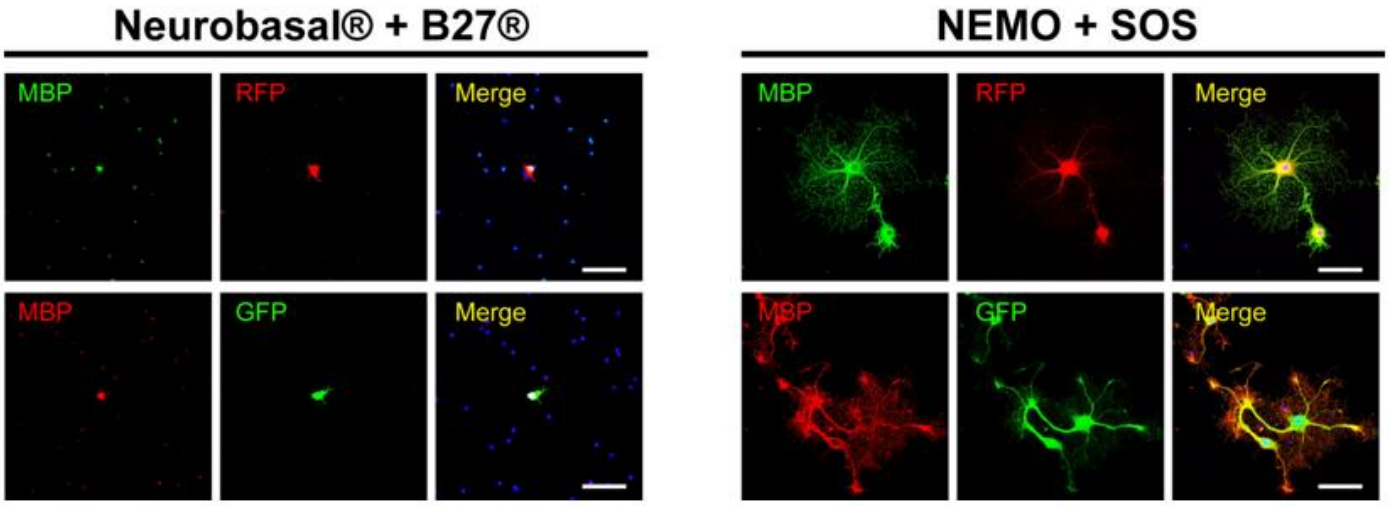

Figure 2. Live cell fluorescent imaging of glial cells. Successful FACs sorting, culturing and differentiation of viable GFP and RFP labelled oligodendrocyte progenitor cells (OPCs) from adult mouse brain is only achievable with NEMO and SOS compared to Neurobasal® and B27®.

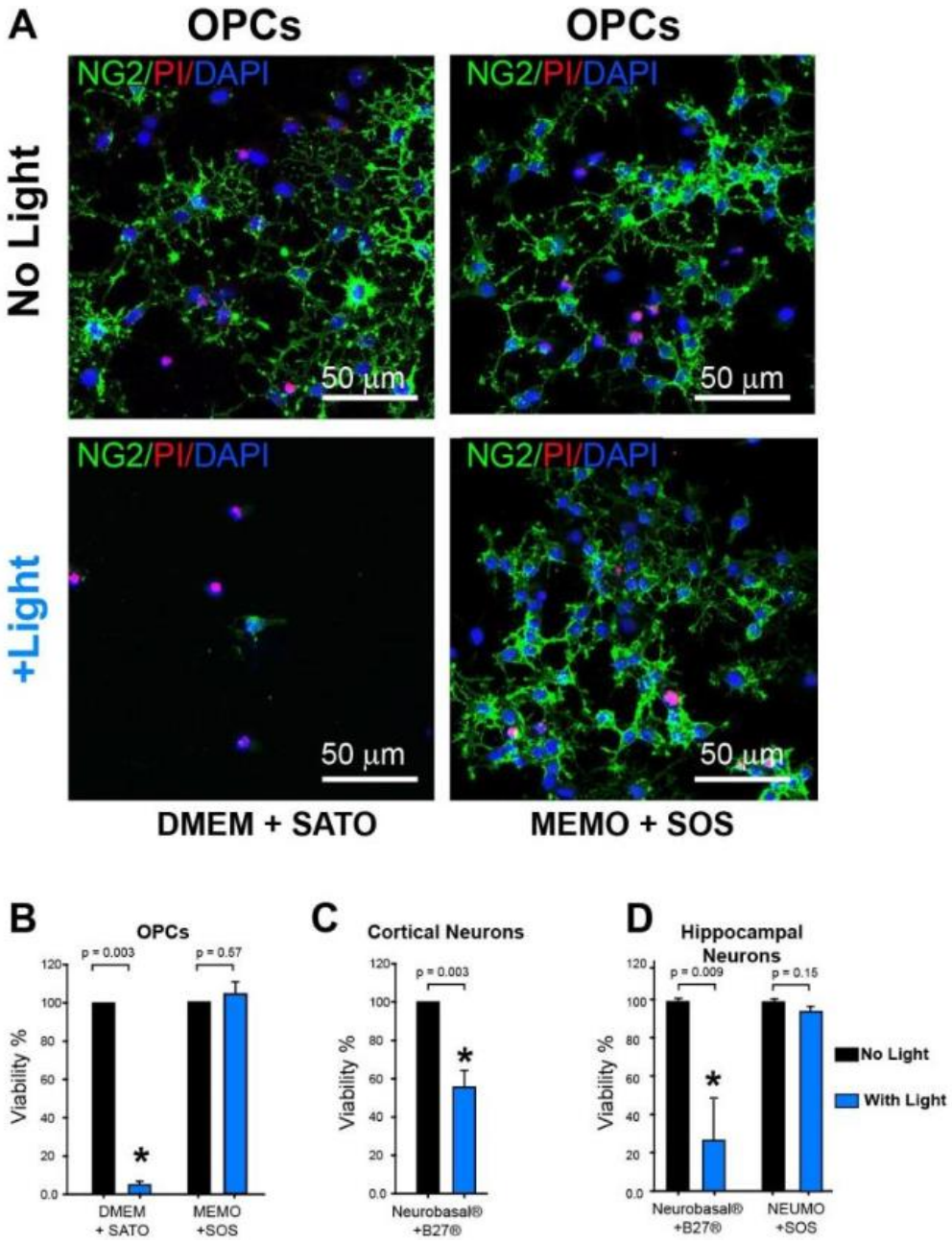

Figure 3. Enhanced live cell fluorescent imaging of OPCs (A,B), Cortical Neurons (C) and Hippocampal Neurons (D) when cultured in BrightCell photostable media (MEMO/SOS) vs standard conditions (DMEM/SOS).

References

1. Ragnhildur T. Káradóttir et. al. Surpassing light-induced cell damage in vitro with novel cell culture media. Nature Scientific Reports. Sci Rep. 2017 Apr 12;7(1):849.

2. Artifacts of Light. Nature Methods (2013) volume 10 (12): 1135.

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