User Guide

LiveLight™

NEUMO®
Photostable Cell Culture Medium

Cat M07

Protocol Version 2.5

Contents

NEUMO® Photostable Cell Culture Medium

Introduction

LiveLight™ is a range of cell culture media and supplements that have been reformulated with specific phototoxic components eliminated or replaced. LiveLight™ cell culture products allow prolonged exposure of cells to light whilst maintaining high levels of cell viability and functionality.

Experiments with cultured cells, including fluorescence microscopy, optogenetics, fluorescence activated cell sorting (FACS) and automated cell culture, often entail high levels or prolonged exposure to 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 issue has been discussed, for example, in "Artifacts of Light", Nature Methods (2013) volume 10 (12) page 1135.

Product Components

The LiveLight™ cell culture system encompasses three different photostable products:

- MEMO® medium replaces DMEM
- NEUMO® medium replaces Neurobasal® media
- SOS® supplement replaces B-27® and similar neuronal supplements (e.g. N2, SATO, NS21)

The specific cell type being used will determine which product or combination of products would be most suitable. As with other media, additional supplements (see table below) may be added to enhance the performance.

Table 1. LiveLight™ range of products.

Product	Catalogue Number	Storage
MEMO® Media, 100 ml	**M06-100**	2 to 8°C
MEMO® Media, 500 ml	**M06-500**	2 to 8°C
NEUMO® Media, 100 ml	**M07-100**	2 to 8°C
NEUMO® Media, 500 ml	**M07-500**	2 to 8°C
SOS® Supplement, 25x, 50 ml	**M09-50**	-20°C

NEUMO®

NEUMO® is a photostable medium which allows manipulation and imaging of cells in light. It has been specifically developed for the culture of neuronal cells. Use of NEUMO® supplemented with SOS® during exposure to prolonged periods of light results in significantly higher levels of cell viability. NEUMO® has been formulated to allow growth of a range of neuronal cells and can therefore be used as a replacement for Neurobasal® just prior to and during exposure to light. During other periods of culture in dark conditions, standard Neurobasal® should be used.

Supplementation of NEUMO®

As with Neurobasal®, the optimal supplementation requirements of NEUMO® depend on the cell type. Recommended medium supplementation for non-neuronal cells and neuronal cells are shown on Table 2 and 3 on page 5.

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 Neurobasal® as well as with NEUMO®.

Table 2. Examples of media supplements for non-neuronal cells.

Component	Catalogue Number (Supplier)	Stock*	Final concentration	Amounts required (for 100 ml)	Amounts required (for 500 ml)
SOS®	M09 (*Cell Guidance Systems*)	25x	1x	4 ml	20 ml
Human Recombinant Insulin	12585-014 (*Life Technologies*)	4 mg/ml	15 µg/ml	375 µl	1875 µl
Glutamax	35050-087 (*Life Technologies*)	100x	1x	1 ml	5 ml
Supplement A	M10 (*Cell Guidance Systems*) Provided free of charge with LiveLight™	9.9% w/v	0.099% w/v	1 ml	5 ml

Table 3. Examples of media supplements for neuronal cells.

Component	Catalogue Number (Supplier)	Stock*	Final concentration	Amounts required (for 100 ml)	Amounts required (for 500 ml)
SOS®	M09 (*Cell Guidance Systems*)	25x	1x	4 ml	20 ml
Human Recombinant Insulin	12585-014 (*Life Technologies*)	4 mg/ml	15 µg/ml	375 µl	1875 µl
Glutamax	35050-087 (*Life Technologies*)	100x	1x	1 ml	5 ml
T3 (3,3′′,5-Triiodo-L-thyronine Sodium salt)	T6397 (*Sigma*)	2 mg/ml in 0.1 M NaOH	0.4 µg/ml in 20 µM	20 µl	100 µl
T4 (L-thyroxine)	T1775 (*Sigma*)	2 mg/ml in 0.1M NaOH	0.4 µg/ml in 20 µM	20 µl	100 µl

*Stock multiples, where given, are approximate

Protocol

A. To prepare medium

1. Thaw SOS® at 37°C. SOS® is supplied as a 25 x concentrate and should be added to NEUMO® to a final concentration as shown in Table 2 and Table 3. Any liquid remaining should be aliquoted into working volumes and store at -20°C. **Avoid freeze-thawing of SOS® supplement more than twice.**

2. Add additional components according to Table 2 shown above.

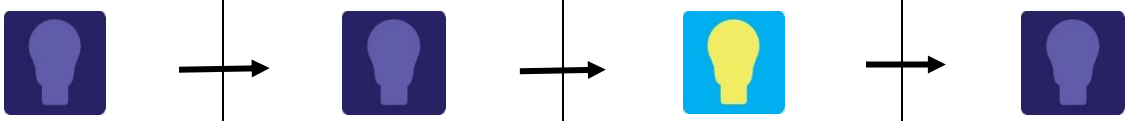
3. Once supplemented, the complete media is stable for 2 weeks when stored at 4°C.

B. Use of NEUMO® and SOS®

Some of the phototoxic components present in standard media (but removed from NEUMO®) contribute to cellular proliferation. Consequently, 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.

Table 4. NEUMO® and SOS® Protocol

Expansion of cells in dark	12-24 hours prior to light exposure	Exposure to light	Following exposure to light
			
Neurobasal®	Neurobasal® removed and replaced with NEUMO®	NEUMO®	NEUMO® removed and replaced with Neurobasal®
SOS®	SOS®	SOS®	SOS®

1. Initially expand/maintain cells in the dark using Neurobasal® supplemented with SOS®.

2. Between 12–24 hours prior to light exposure, replace Neurobasal® + SOS® with pre-warmed NEUMO® + SOS®. Any remnants of Neurobasal® should be washed away by centrifugation. Cells will remain viable in NEUMO® + SOS® for up to 3 days, if required. **Aliquot media to pre-heat, repetitive heating and cooling of bottles of media may cause precipitation.**

3. Perform experiment requiring exposure to light.

4. After experiment, remove the NEUMO®/SOS® and replace with Neurobasal®/SOS®. Any remnants of NEUMO® should be washed away by centrifugation.

Storage & Stability

NEUMO® media should be stored at 2 to 8°C. SOS® supplement and Supplement A should be stored at -20°C for up to 1 year from manufacture. Avoid freeze-thaw cycles. For product stability please refer to the expiry date on the label of the bottle.

Purchaser Notification

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Cell Guidance Systems' reagents and services enable control, manipulation and monitoring of the cell, both *in vitro* and *in vivo*.

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- Purification
- Detection
- Tracking
- NTA Service

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Cell Counting Reagent

Matrix Proteins

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- Photostable
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Gene Knock-Up System

Cytogenetics Analysis

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