User Guide

LiveLight™

SOS®
Cell Culture Supplement

Cat M09

Protocol Version 2.5

Contents

SOS®
Cell Culture Supplement

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

SOS®

SOS® is a photostable supplement which allows manipulation and imaging of cells in light. Use of SOS® with Livelight™ media during exposure to prolonged periods of light results in significantly higher levels of cell viability. As well as its use in optogenetic experiments, SOS® can also be used to directly replace other supplement such as B-27® and SATO in a range of cell culture media throughout experiments. For optimum culturing conditions of non-neuronal cells, SOS® should be used in conjunction with Supplement A.

SOS® Usage

SOS® can be used as a serum free supplement to support both neuronal, and non-neuronal cell types. An example of how to supplement with SOS® to make a complete medium shown on Table 2 and 3 below.

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)
Standard Media		1x	1x	100 ml	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*)	100 x	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)
Standard Media		1x	1x	100 ml	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*)	100 x	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

For supplementation requirements using MEMO® and NEUMO®, please see respective User Guides.

Protocol

A. To prepare medium

1. Thaw SOS® at 37°C. SOS® is supplied as a 25 x concentrate and should be added to your final media 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.**

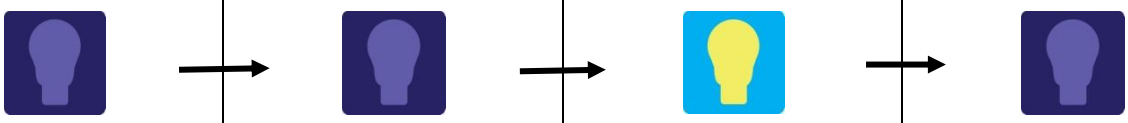
2. Add additional components according to Table 2 and 3 shown above.

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

B. Use of LiveLight™ media and SOS®

Some of the phototoxic components present in standard media (but removed from LiveLight™ media) contribute to cellular proliferation. Consequently, NEUMO® and MEMO® should only be used just prior to and during the phase of the experiment during 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.

Table 4. MEUMO® or NEUMO® and SOS® Protocol

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

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

2. Between 12–24 hours prior to light exposure, replace DMEM + SOS® or Neurobasal® + SOS® with pre-warmed MEMO® + SOS® or NEUMO® + SOS® respectively. Any remnants of DMEM® or Neurobasal® should be washed away by centrifugation. Cells will remain viable in MEMO® + SOS® or 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 MEMO®/SOS® or NEUMO®/SOS® and replace with DMEM/SOS® or Neurobasal®/SOS® respectively. Any remnants of MEMO® or NEUMO® should be washed away by centrifugation.

Storage & Stability

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*.

Growth Factors
- Recombinant
- Sustained Release

Exosomes
- Purification
- Detection
- Tracking
- NTA Service

Small Molecules

Cell Counting Reagent

Matrix Proteins

Cell Culture Media
- Pluripotent Stem Cells
- Photostable
- *In Vitro* Blastocyst Culture
- ETS-embryo Culture
- Custom Manufacturing Service

Gene Knock-Up System

Cytogenetics Analysis

General info@cellgs.com
Technical Enquiries tech@cellgs.com
Quotes quotes@cellgs.com
Orders order@cellgs.com

www.cellgs.com

EUROPE
Cell Guidance Systems Ltd
Maia Building
Babraham Bioscience Campus
Cambridge
CB22 3AT
United Kingdom
T +44 (0) 1223 967316
F +44 (0) 1223 750186

USA
Cell Guidance Systems LLC
Helix Center
1100 Corporate Square Drive
St. Louis
MO 63132
USA
T 760 450 4304
F 314 485 5424