User Guide

LiveLight™

MEMO®
Photostable Cell Culture Medium

Cat M06

Protocol Version 2.5

Contents

MEMO® Photostable Cell Culture Medium

Introduction

LiveLight™ is a range of cell culture media and supplements that have been reformulated to eliminate or replace specific phototoxic components. 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 require high-intensity light or prolonged exposure to light. However, cell culture media and supplements contain components that are converted to toxic free radicals by light. In particular, Dulbecco's Modified Eagle Medium (DMEM) and Neurobasal® medium, as well as supplements such as B-27®, SATO and NS21, can lead to significant perturbation of cellular behavior and a marked increase in cell death.[1]

Product Components

The LiveLight™ cell culture system (see Table 1) 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 and 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 may be required (see Table 2).

Use of MEMO® supplemented with SOS® during prolonged exposure to light results in significantly higher levels of cell viability. SOS® is a photostable, serum-free, neuronal/stem cell supplement that maintains an essential level of proteins needed for cell culture with potent antioxidants. SOS® has been designed to directly replace phototoxic serum-free supplements such as B-27®, NS21, SATO and N2. SOS® can be used with standard DMEM as well as with MEMO®, and must be used for the entire culture period.

1. Artifacts of light. Nature Methods December 2013;10:1135

Table 1. LiveLight™ product range

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

MEMO®

MEMO® is a photostable medium which allows for manipulation and imaging of cells in light. MEMO® replaces DMEM prior to, and during, exposure to light. Standard DMEM should be used when culturing cells in dark conditions.

Supplementation of MEMO®

As with DMEM, the optimal supplementation requirements of MEMO® depend on the cell type. Recommended medium supplementation for non-neuronal cells and neuronal cells are shown overleaf in Table 2 and Table 3.

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 as well as with MEMO®.

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

Component	Catalogue Number (Supplier)	Stock concentration*	Final concentration	Volume required (in 100 ml)	Volume required (in 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
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 concentration*	Final concentration	Volume required (in 100 ml)	Volume required (in 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
T3	T6397 (*Sigma*)	2 mg/ml in 0.1 M NaOH	0.4 µg/ml in 20 µM	20 µl	100 µl
T4	T1775 (*Sigma*)	2 mg/ml in 0.1 M 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 25x concentrate and should be added to MEMO® at a final concentration as shown in Table 2 and Table 3. Any liquid remaining should be aliquoted into working volumes and stored at -20°C. **Avoid freeze-thawing of SOS® supplement more than twice.**

2. Add any additional media components according to Table 2 or Table 3, as shown above.

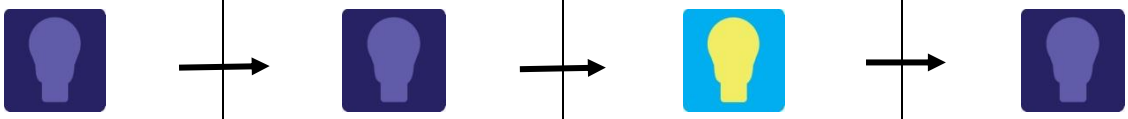
3. Once supplemented, the complete media is stable for 2 weeks when stored at 4°C.

B. Use of MEMO® and SOS®

Some of the phototoxic components present in standard media, which are not present in MEMO®, contribute to cellular proliferation. Consequently, MEMO® should only be used just prior to and during the experimental phase when cells are exposed to prolonged periods of light. In contrast, SOS® does not affect cellular viability and proliferation, and therefore should be used continuously during cell maintenance.

SOS® should replace neuronal supplements, such as B-27®, if these are required in your media. If FBS is used as a supplement in your media, 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. MEMO® and SOS® Protocol

Expansion of cells in dark	12–24 hours prior to light exposure	Exposure to light	After light exposure
			
DMEM + SOS®	Remove DMEM + SOS® and replace with MEMO® + SOS®	MEMO® + SOS®	Remove MEMO® + SOS® and replace with DMEM + SOS®

1. Expand and maintain cells in the dark using DMEM supplemented with SOS®.

2. Between 12–24 hours prior to light exposure, replace DMEM + SOS® with pre-warmed MEMO® + SOS®. Any remnants of DMEM should be washed away by centrifugation. Cells will remain viable in MEMO® + SOS® for up to 3 days, if required.
Aliquot media prior to warming to 37°C. Repetitive heating and cooling of media may cause precipitation.

3. Perform experiment requiring exposure to light.

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

Storage & Stability

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

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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

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