LiveLight™

MEMO®

Photostable Cell Culture Medium

User's Guide

Version 1.2

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 behaviour 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.

Components of the LiveLight™ culture system

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.

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

Storage conditions

MEMO® 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.

MEMO®

MEMO® is a photostable medium which allows manipulation and imaging of cells in light. Use of 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. During other periods of culture in dark conditions, standard DMEM should be used.

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 as follows:

Cell type	*Component*	*Catalog Number (Supplier)*	*Stock**	*Amounts required (for 100 ml)*	*Amounts required (for 500 ml)*
Non-neuronal cells	MEMO®	M06 (*Cell Guidance Systems*)	1 x	100 ml	500 ml
	SOS®	M09 (*Cell Guidance Systems*)	25 x	4 ml	20 ml
	Human Recombinant Insulin	12585-014 (*Life Technologies*)	4 mg/ml	375 µl	1875 µl
	Supplement A	M10 (*Cell Guidance Systems*) Provided with LiveLight™ products	9.9% w/v	1 ml	5 ml
Neuronal cells	MEMO®	M06 (*Cell Guidance Systems*)	1 x	100 ml	500 ml
	SOS®	M09 (*Cell Guidance Systems*)	25 x	4 ml	20 ml
	Human Recombinant Insulin	12585-014 (*Life Technologies*)	4 mg/ml	375 µl	1875 µl
	T3 (3,3″,5-Triiodo-L-thyronine Sodium salt)	T6397 (*Sigma*)	2mg/ml in 0.1 M NaOH	25 µl	100 µl
	T4 (L-thyroxine)	T1775 (*Sigma*)	2mg/ml in 0.1M NaOH	25 µl	100 µl

Table 1. Supplementation requirements (*stock multiples, where given, are approximate).

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®.

Protocol

To prepare medium

1. Thaw SOS® at 37°C. SOS® is supplied as a 25 x concentrate and should be added to MEMO® to a final concentration of 4%. Any liquid remaining should be aliquoted into working volumes and store at -20°C.
    a. *Avoid freeze-thawing of SOS® supplement more than twice.*
2. Add additional components according to Table 1 shown above.
3. Once supplemented, the complete media is stable for 2 weeks when stored at 4°C.

Use of MEMO® and SOS®

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

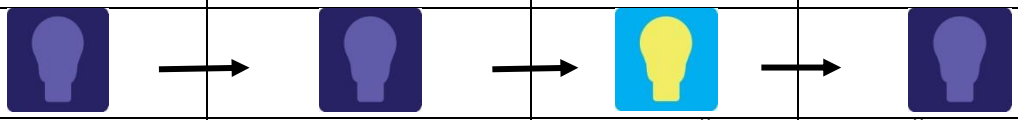
Expansion of cells in dark	12-24 hours prior to light exposure	Exposure to light	Following exposure to light
			
DMEM	DMEM removed and replaced with MEMO®	MEMO®	MEMO® removed and replaced with DMEM
SOS®	SOS®	SOS®	SOS®

Figure 1. DMEM should be replaced with MEMO® prior to and during exposure to light. SOS® should be used throughout the experiment.

1. Initially expand/maintain cells in the dark using DMEM supplemented with SOS®.
2. The evening before exposure to prolonged periods of light, replace DMEM/SOS® with pre-warmed MEMO®/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®/SOS® and replace with DMEM/SOS®.