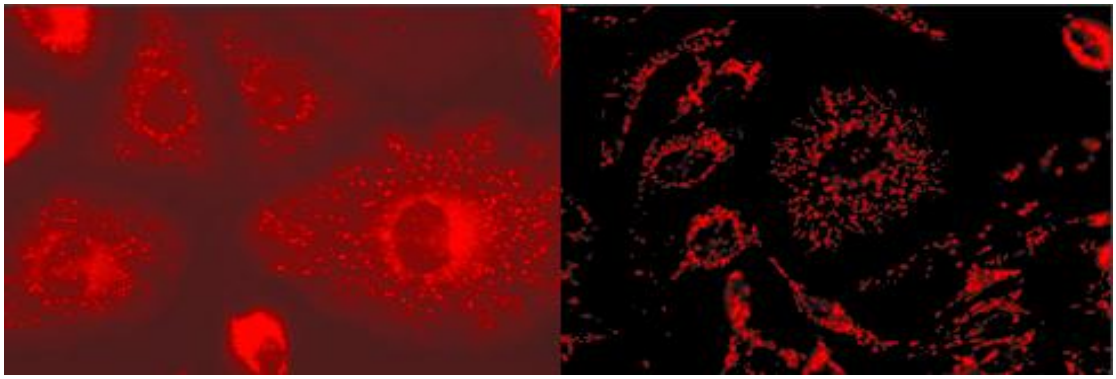
PRODUCT INFORMATION SHEET



MARKERGENE™ OPTI-KLEAR™ LIVE CELL IMAGING BUFFER Product M1898

MARKER GENE TECHNOLOGIES, INC.
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I. OVERVIEW

Physiology, the study of living tissues, has gained wide spread application in microscopy with the advent of fluorescent dyes and inverted microscopes dedicated to their viewing. A critical component is maintaining physiological conditions in media formulations, including pH and osmolarity, without contributing to background fluorescence. Most cells are grown in media formulations that use bicarbonate buffers, requiring the continuous presence of CO_2 to maintain physiological pH. Just 30 minutes outside a CO_2 incubator can change the pH from 7.3 to 7.8, and to 8.5 after an hour.

Many of these formulations include phenol red which can have estrogenic effects, and quench fluorescence signals. Most media formulations also contain autofluorescent components like riboflavin and folic acid, that while quenched when phenol red is present, will autofluoresce when phenol red-free solutions are used. And while phenol red itself is not fluorescent, there may be contaminants present in the dye that are.

Marker Gene Technologies, Inc. now offers HEPES based **MarkerGene™Opti-Klear™ Live Cell Imaging Buffer** as an ideal substitute for growth media for all extended live cell imaging sessions.

HEPES has a pKa of 7.66 making it a much stronger buffer in the pH 7.2-7.6 range than media formulations based on bicarbonate buffer. Unlike the bicarbonate buffering system that requires the use of a CO_2 incubator, the HEPES buffering system may be used with or without a CO_2 atmosphere.

Shipped in amber Nalgene bottles to prevent ambient light-induced toxicity, the sterile filtered **MarkerGene™Opti-Klear™ Live Cell Imaging Buffer** is formulated to:

i) Maintain proper pH and osmolarity for hours
ii) Lower background fluorescence
iii) Provide energy source and preserve fluorescent signals

II. MATERIALS

MarkerGene™Opti-Klear™ Live Cell Imaging Buffer:
30 mL of 5X stock. pH 7.5

Storage and Handling.

Store HEPES based **MarkerGene™Opti-Klear™ Live Cell Imaging Buffer** in the amber Nalgene bottle provided to avoid creation of toxic hydrogen peroxide which can occur when HEPES based buffers are exposed to ambient light for long periods. Solution is sterile filtered – and should be stored at 4^{o}C or, for extended periods, stored frozen (-20°C). In case of contact with skin or eyes, wash thoroughly with soap and cold-water. Solution should be stable for at least 6 months following purchase.

III. PROTOCOL:

This bottle provides sufficient reagent to make 150 mL of imaging solution.

Standard protocol:

1. To prepare 10 mL of 1X imaging solution, combine 2mL of 5X **MarkerGene™Opti-Klear™ Live Cell Imaging Buffer** with 8 mL sterile water and briefly mix.

2. Remove media, wash cells 1-2 times with sterile PBS (if desired), and replace with 1X **MarkerGene™Opti-Klear™ Live Cell Imaging Buffer.**

3. Proceed to imaging as normal.

FIGURES:

Figure 1: Reduction of background fluorescence by switching from DMEM with 10% fetal calf serum (Fisher) to **MarkerGene™Opti-Klear™ Live Cell Imaging Buffer**. HeLa cells were treated with JC-1 (Marker Gene Technologies, Inc. Product M1842) for 60 minutes to detect J-aggregate formation in healthy, polarized mitochondria and imaged with a 40X oil objective and photographed with identical exposure times and light intensity.

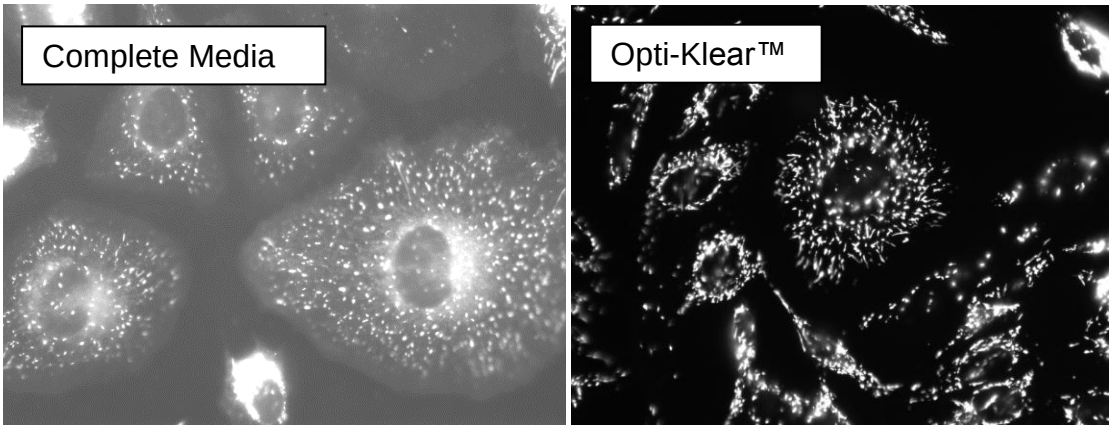


Figure 2: Reduction of background fluorescence by switching from DMEM with 10% fetal calf serum (Fisher) to **MarkerGene™Opti-Klear™ Live Cell Imaging Buffer**. HeLa cells were treated with JC-1 (Product M1842) for 60 minutes to detect total mitochondria in healthy, polarized mitochondria and imaged with a 40X oil objective and photographed with identical exposure times and light intensity.

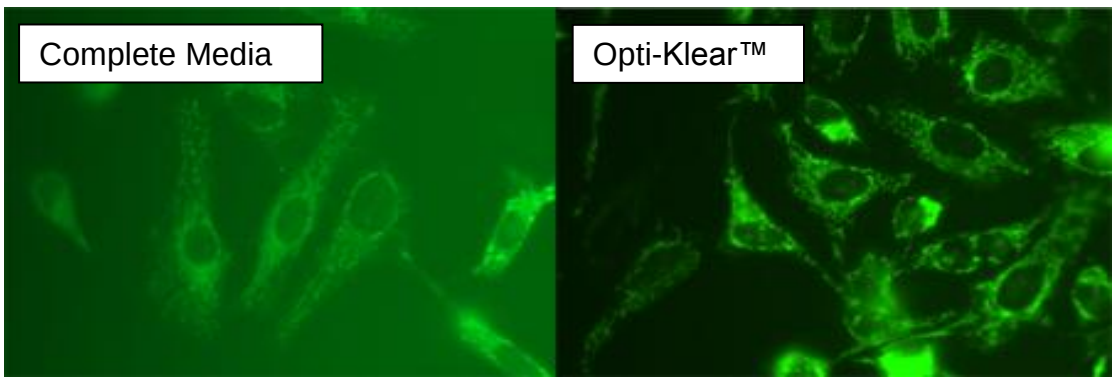
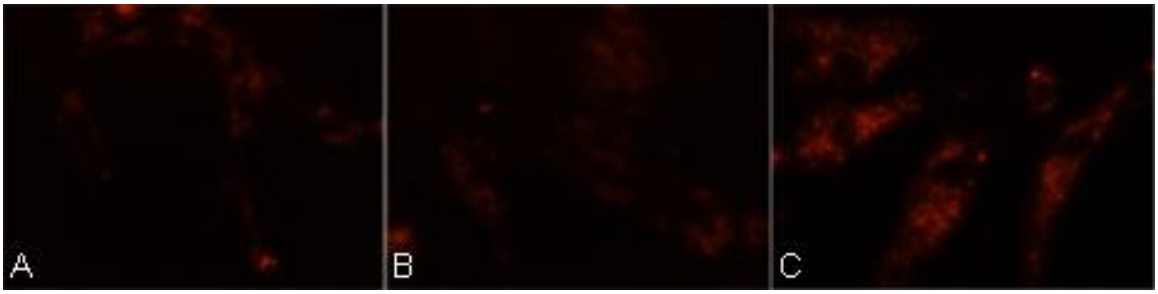


Figure 3: AG06173 Normal Human Fibroblasts were stained with LysoTracker™ (Life Technologies) for 1 hour then washed 3X with PBS. Media was replaced with A) complete media B) serum-free media C) **MarkerGene™Opti-Klear™ Live Cell Imaging Buffer** and imaged with 40X dry long working distance objective and identical exposure times and light intensity.



PRODUCT M1898

DESCRIPTION	QUANTITY	PART NO.	STORAGE
SAMPLE PREPARATION			
MarkerGene™Opti-Klear™ Live Cell Imaging Buffer - sterile filtered	30 mL	M1898	C, L
DOCUMENTATION			
MSDS SHEETS	1		
PRODUCT INFORMATION SHEET	1		

Notes: C=store cold (4°C); L=light sensitive

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NOTES:

Contact and Support

For questions or comments on this or any product from Marker Gene Technologies, Inc., you may contact us by phone or via our website. We welcome customer feedback and we make every effort to improve our products based on input from our clients.

To ask a question or make a comment or suggestion, you can call us at **1-888-218-4062** or fax to **541-342-1960**.

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We want to thank you for your purchase and hope that you will continue to order from Marker Gene Technologies, Inc.

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