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ab275939 Opti-Klear™ Live Cell Imaging Buffer, 5X

View Opti-Klear™ Live Cell Imaging Buffer, 5X:

www.abcam.com/ab275939

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Growth media substitute for extended live cell imaging sessions.

This product is for research use only and is not intended for diagnostic use.

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1. 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 15 minutes outside a CO_2 incubator can change the pH from 7.4 to 8.1, and to 8.7 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.

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 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

2. Materials Supplied and Storage

Store HEPES based 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°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.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temp
Opti-Klear™ Live Cell Imaging Buffer	30 mL	4°C

3. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

4. 1X Buffer Preparation

This bottle provides sufficient 5X buffer to make 150 mL of imaging solution.

Standard protocol:

1. To prepare 10 mL of 1X imaging solution, combine 2mL of 5X 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 Opti-Klear™ Live Cell Imaging Buffer.
3. Proceed to imaging as normal.

5. Images

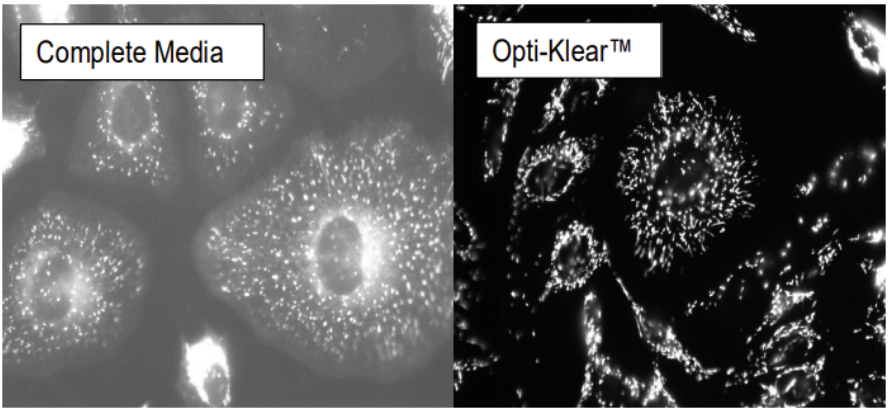

Figure 1. Reduction of background fluorescence by switching from DMEM with 10% fetal calf serum to Opti-Klear™ Live Cell Imaging Buffer. HeLa cells were treated with JC-1for 60 minutes to detect J-aggregate formation in healthy, polarized mitochondria and imaged with a 40X oil objective and photographed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

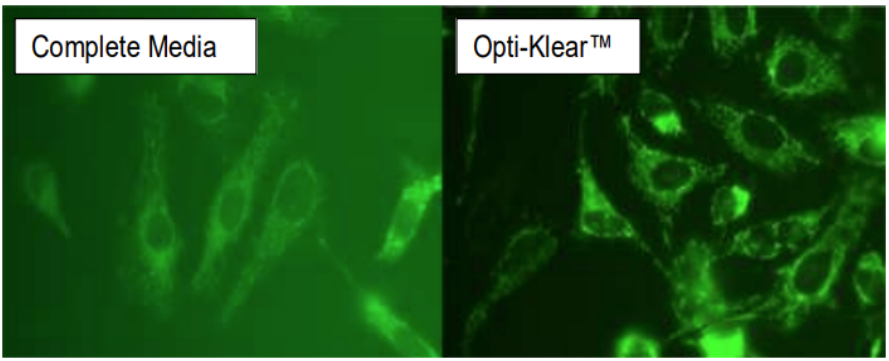

Figure 2. Reduction of background fluorescence by switching from DMEM with 10% fetal calf serum to Opti-Klear™ Live Cell Imaging Buffer. HeLa cells were treated with JC-1for 60 minutes to detect total mitochondria in healthy, polarized mitochondria and imaged with a 40X oil objective and photographed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

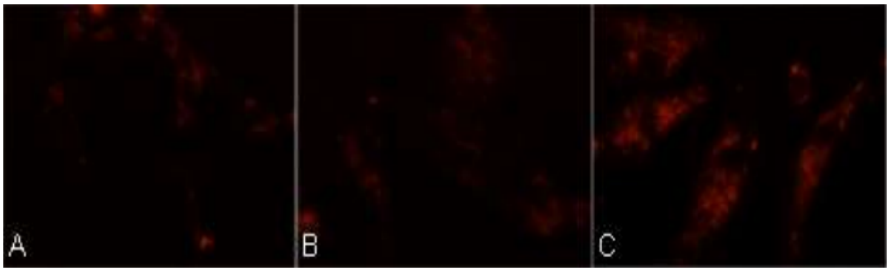

Figure 3. Normal Human Fibroblasts were stained with LysoTracker™ for 1 hour then washed 3X with PBS. Media was replaced with A) complete media B) serum-free media C) Opti-Klear™ Live Cell Imaging Buffer and imaged with 40X dry long working distance objective and identical exposure times and light intensity.

Technical Support

For all technical or commercial enquiries please go to:

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www.abcam.co.jp/contactus (Japan)