Chromeo™ Red Fluorescent Fixed Cell Staining Kit

(version A1)

Catalog No. 15006

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Overview

The Chromeo™ Red Fluorescent Fixed Cell Staining Kit utilizes a proprietary dye that functions as a cellular stain to label the nuclear membrane(s), mitochondria, fibers and nucleoli in fixed cells. This unique dye is not fluorescent until after it has reacted with an amino group, which induces a structural change resulting in bright red fluorescence. It can be excited between 520 nm and 550 nm (maximum at 540 nm) by commonly used excitation sources and standard filter sets of fluorescent microscopes or readers. Another principal characteristic of this fluorescent dye is its long Stokes shift of about 100 nm, with an emission maximum at 627 nm.

To selectively stain the nuclei of fixed cells, MAXfluor™ DAPI Mounting Medium stain is also included in the kit. The combination of cellular and nuclear staining can be used to observe changes in the shape and localization of the nucleus, to visualize changes in cellular morphology and to monitor cell-cell contacts in a culture system.

In addition, MAXfluor™ DAPI Mounting Medium provides optimal fluorescence stability, superior anti-fading during long-term storage, and inhibition of photobleaching during examination by both traditional (single photon excitation) and super-resolution microscopy (4Pi).

Multi-color cell staining in combination with FITC or other green fluorescent molecules

This kit is ideal for use as a counterstain of cells in direct or indirect immunofluorescence experiments in combination with FITC or other 488 nm excitable fluorescent molecules. Target proteins labeled with green fluorescent dyes can easily be localized because the long Stokes shift of the cellular stain separates its emission spectrum from the emission spectra of GFP and 488 nm excitable dyes. This facilitates co-staining experiments without any interference or overlap between the red and green signals. The DAPI stain, with 358 nm excitation and 461 nm emission maxima, can serve as a nuclear counterstain.

product	format	catalog no.
Chromeo™ Red Fluorescent Fixed Cell Staining Kit	1 kit	15006

Chromeo™ Red Fluorescent Fixed Cell Staining Kits are for research use only. Not for use in diagnostic procedures.

Kit Performance

A typical multi-color cell staining experiment is shown below; the cellular and nuclear stains of the Chromeo Red Fluorescent Fixed Cell Staining Kit are used in combination with alpha tubulin monoclonal antibody that is visualized by Chromeo 488 secondary antibody conjugate.

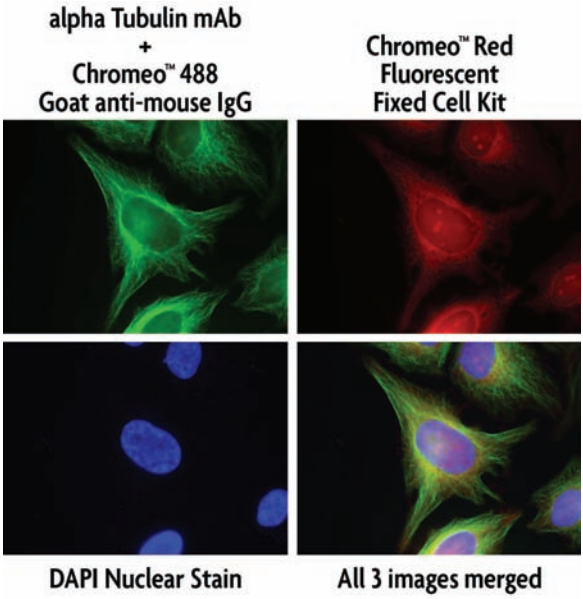

Figure 1: Staining of fixed HeLa cells using the Chromeo Red Fluorescent Fixed Cell Staining Kit and Chromeo 488. HeLa cells were stained with alpha Tubulin mouse mAb (Clone 5-B-1-2, Catalog No. 39527) and Chromeo 488 Goat anti-mouse IgG (Catalog No. 15031). The Chromeo Red Fluorescent Fixed Cell Staining Kit was used for counterstaining the cellular structures (red) and the nuclei (blue).

Kit Components and Storage

Please store each component at the temperature indicated in the table below and protect from light, if indicated.

Reagents	Quantity	Storage / Stability
Chromeo™ Red Fluorescent Fixed Cell Stain, lyophilized powder	1 vial	-20°C for 6 months in the dark
Dimethylformamide (DMF)	1 vial	4°C for 6 months
MAXfluor™ DAPI Mounting Medium	2 x 2 ml	4°C for 6 months in the dark

Additional materials required

- Tissue culture supplies
- 100% methanol for fixation
- Fluorescence instrumentation
- PBS

Optional materials for immunofluorescence applications

- Blocking solution; MAXblock™ Blocking Medium (Catalog Number 15252) is recommended
- Washing solution; MAXwash™ Washing Medium (Catalog Number 15254) is recommended

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Note: Dilutions and quantities provided are guidelines. You may need to vary conditions to obtain optimal results in your specific system.

A. Preparation of the Fixed Cell Stain Stock Solution

To prepare the Stock Solution, add 100 µl DMF into the vial containing the Chromeo Red Fluorescent Fixed Cell Stain and mix carefully with the pipette tip. This 5 mM stock solution, which will have a purple color, should be stored in aliquots at -20°C in the dark prior to use. Please avoid repeated freeze-thaw cycles.

B. Preparation of the Staining Solution

Dilute the appropriate volume of Chromeo Red Fluorescent Fixed Cell Stain Stock Solution in PBS. Generally a 1:1000 - 1:5000 dilution results in the best cellular staining, but optimization of the concentration may be required for different cell lines or primary cells.

If the stain is used as a counterstain in multi-color immunofluorescence experiments, the cellular stain can be prepared directly in the incubation solution of the secondary antibody.

Please note that the kit is designed to stain 260 slides, based on the use of 22 mm square coverslips. If the optimal dilution of the fixed cell stain allows more staining reactions, additional MAXfluor DAPI Mounting Medium may be purchased separately.

The table below shows the volume of dye available depending on the chosen concentration, and the resulting number of experiments that can be performed in different cell culture systems:

Dye Concentration (µM)	Final Volume Available (ml)	Number of wells using a 6-well plate (1 ml/well)	Number of wells using 8-well chambered slides (200 µl/well)	Number of wells using a 96-well plate (100 µl/well)	Number of wells using a 384-well plate (50 µl/well)
1	500	500	2500	5000	10000
2	250	250	1250	2500	5000
3	166	166	830	1660	3320
4	125	125	625	1250	2500
5	100	100	500	1000	2000

C. Cell Staining

Note: To ensure the quality of the staining and to maintain the stability of the dye, minimize light exposure of the stained cells as much as possible.

Fixed Cells – Cell stain only

1. Grow cells to the desired confluence and wash twice with cold PBS.
2. Fix cells by adding 100% ice-cold methanol and placing the plate at -20°C for 10 minutes.
3. Wash cells twice with PBS.
4. Add the appropriate amount of diluted Cell Stain Solution (in PBS) to cells and incubate for 30 minutes at room temperature. Protect from light during the incubation.
5. Wash cells twice with PBS, then image.

Fixed cells on coverslips should be mounted on glass slides prior to imaging. MAXfluor DAPI Mounting Medium is designed to be dispersed over the entire coverslip. The recommended volume is 15 µl per 22 mm square coverslip. The coverslip may be sealed with nail polish or other sealants for long-term storage.

Fixed Cells – Cell stain in multi-color applications

1. Grow cells to the desired confluence and wash twice with cold PBS.
2. Fix cells by adding 100% ice-cold methanol and placing the plate at -20°C for 10 minutes.
3. Wash cells twice with PBS.
4. Block the fixed cells.
5. Add primary antibody and incubate at room temperature.
6. Wash cells twice with PBS
7. Add the combined secondary antibody / Cell Stain Stock Solution to cells and incubate for 30 minutes at room temperature. Protect from light during the incubation.
8. Wash cells twice with PBS, then image.

Fixed cells on coverslips should be mounted on glass slides prior to imaging. MAXfluor DAPI Mounting Medium is designed to be dispersed over the entire coverslip. The recommended volume is 15 µl per 22 mm square coverslip. The coverslip may be sealed with nail polish or other sealants for long-term storage.

Note: The effectiveness of MAXfluor Mounting Medium may depend on which fluorescent dye it is used with. It has been optimized for, and shown to increase the fluorescence stability of, Fluorescein and Chromeo 488. MAXfluor is not recommended for use with Alexa 488, as it has been shown to reduce the dye's intensity in widefield microscopy.

D. Image Acquisition

Analyze the stained cells by fluorescence microscopy. To detect the DAPI-stained nuclei, a standard DAPI filter set (370-410 nm/435-485 nm) can be used. To detect the cellular stain in combination with DAPI and FITC, GFP or other 488-excitable dyes, a commonly used Cy3 filter set (550-580 nm/590-650 nm) will separate the fluorescent spectra.

In general, the broad absorption and emission peaks of the cellular stain allow much flexibility for detection and the choice of commonly used filter sets.

Appendix

Section A. Troubleshooting Guide

Problem/question	Possible cause	Recommendation
Staining differs between different cell types	The amount of stain necessary to get optimal staining may vary between different cell types.	Optimize the staining experiment by using different dilutions of the dye stock solution.
Background staining present	The concentration of the cellular stain is too high.	Dilute the dye stock solution further.
Intensity of the staining is dim	Fluorescent instability	Use MAXfluor Mounting Medium to prevent photobleaching of the dyes and stain.
Background staining present in IF experiments	Non-specific binding of primary antibody	Use MAXblock and MAXwash media to reduce non-specific binding.

Section B. Related Products

Fluorescent Cell Stains	Format	Catalog No.
LavaCell™ Live Cell Membrane Staining	200 µg	15004
Chromeo™ Live Cell Mitochondrial Staining Kit	1 kit	15005

Fluorescent Dyes	Excitation / Emission	Format	Catalog No.
Chromeo™ 488 Carboxylic Acid	488 nm / 517 nm	1 mg	15510
Chromeo™ 488 NHS-Ester	488 nm / 517 nm	1 mg	15511
Chromeo™ 494 Carboxylic Acid	494 nm / 628 nm	1 mg	15110
Chromeo™ 494 NHS-Ester	494 nm / 628 nm	1 mg	15111
Chromeo™ 505 Carboxylic Acid	505 nm / 526 nm	1 mg	15610
Chromeo™ 505 NHS-Ester	505 nm / 526 nm	1 mg	15611
Chromeo™ 546 Carboxylic Acid	545 nm / 561 nm	1 mg	15210
Chromeo™ 546 NHS-Ester	545 nm / 561 nm	1 mg	15211
Chromeo™ 642 Carboxylic Acid	642 nm / 660 nm	1 mg	15310
Chromeo™ 642 NHS-Ester	642 nm / 660 nm	1 mg	15311

Antibody/Protein Labeling	Excitation / Emission	Format	Catalog No.
Chromeo™ 488 Antibody Labeling Kit	488 nm / 517 nm	1 kit	15090
Chromeo™ 494 Antibody Labeling Kit	494 nm / 628 nm	1 kit	15091
Chromeo™ 546 Antibody Labeling Kit	545 nm / 561 nm	1 kit	15092
Chromeo™ 642 Antibody Labeling Kit	642 nm / 660 nm	1 kit	15093

Fluorescent Secondary Antibodies	Format	Catalog No.
Chromeo™ 488 Goat anti-Mouse IgG	1 mg	15031
Chromeo™ 488 Goat anti-Rabbit IgG	1 mg	15041
Chromeo™ 494 Goat anti-Rabbit IgG	1 mg	15042
Chromeo™ 546 Goat anti-Mouse IgG	1 mg	15033
Chromeo™ 546 Goat anti-Rabbit IgG	1 mg	15043
Chromeo™ 642 Goat anti-Mouse IgG	1 mg	15034
Chromeo™ 642 Goat anti-Rabbit IgG	1 mg	15044
ATTO 594 Goat anti-Mouse IgG	250 µl	15037
ATTO 594 Goat anti-Rabbit IgG	250 µl	15047
ATTO 647N (STED) Goat anti-Mouse IgG	250 µl	15038
ATTO 647N (STED) Goat anti-Rabbit IgG	250 µl	15048
ATTO 655N (STED) Goat anti-Mouse IgG	250 µl	15039
ATTO 655N (STED) Goat anti-Rabbit IgG	250 µl	15049

MAX Stain™ Immunofluorescence Tools	Format	Catalog No.
MAXpack™ Immunostaining Media Kit (contains 1 each of 15252, 15253 & 15254)	1 kit	15251
MAXblock™ Blocking Medium	150 ml	15252
MAXbind™ Staining Medium	250 ml	15253
MAXwash™ Washing Medium	1000 ml	15254

Fluorescent Cell Viability Assay	Format	Catalog No.
ToxCount™ Cell Viability Assay	20 x 96 rxns	18010

Fluorescent Protein Labeling	Format	Catalog No.
LigandLink™ pLL-1 Kit	1 kit	34001
LigandLink™ pLL-1-NFκB p65 Kit	1 kit	34004
LigandLink™ pLL-1-p53 Kit	1 kit	34005
LigandLink™ pLL-1-STAT1 Kit	1 kit	34006
LigandLink™ Fluorescein Label	300 rxns	34101
LigandLink™ Hexachlorofluorescein Label	300 rxns	34104

Luciferase Assays	Format	Catalog No.
RapidReporter™ Gaussia Luciferase Assay	100 rxns	33001
	1000 rxns	33002
RapidReporter™ pRR-High vector	10 µg	33003
RapidReporter™ pRR-High Assay	100 rxns	33004
RapidReporter™ pRR-Low vector	10 µg	33005
RapidReporter™ pRR-Low Assay	100 rxns	33006
RapidReporter™ pRR-High-CRE vector	10 µg	33007
RapidReporter™ pRR-High-CRE Assay	100 rxns	33008
RapidReporter™ pRR-High-GR vector	10 µg	33011
RapidReporter™ pRR-High-GR Assay	100 rxns	33012
RapidReporter™ pRR-High-IRF-1 vector	10 µg	33017
RapidReporter™ pRR-High-IRF-1 Assay	100 rxns	33018
RapidReporter™ pRR-High-NFκB vector	10 µg	33009
RapidReporter™ pRR-High-NFκB Assay	100 rxns	33010
RapidReporter™ pRR-High-STAT1 vector	10 µg	33015
RapidReporter™ pRR-High-STAT1 Assay	100 rxns	33016
RapidReporter™ pRR-High-STAT3 vector	10 µg	33013
RapidReporter™ pRR-High-STAT3 Assay	100 rxns	33014

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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