CellMask™ Actin Tracking Stains

Catalog Numbers A57243, A57244, A57245, A57246, A57247, A57248, and A57249

Pub. No. MAN0019419 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ CellMask™ Actin Tracking Stains provide fluorescent staining of polymerized/Filamentous Actin (F-Actin) in live cells or fixed cells. The stains are designed to readily permeate live cells, which provides uniform and specific staining of F-Actin. Live cells stained with CellMask™ Actin Tracking Stains can be fixed for mutiplexability in immunofluorescence (IF)/immunocytochemistry (ICC)/immunohistochemistry (IHC) protocols. For flexibility in experimental designs, CellMask™ Actin Tracking Stains are provided in three colors (Figure 1).

- CellMask™ Green Actin Tracking Stain can be detected using a traditional FITC/GFP filter setting (Ex 503 nm/Em 512 nm)
- CellMask™ Orange Actin Tracking Stain can be detected with TRITC/RFP traditional filter setting (Ex 545 nm/Em 570 nm)
- CellMask™ Deep Red Actin Tracking Stain can be detected with Cy5™/Deep Red traditional filter setting (Ex 652 nm/Em 669 nm)

CellMask™ Actin Tracking Stains detect F-Actin without staining monomeric globular Actins (G-Actin) by using a targeting molecule that closely resembles Jasplakinolide. While easily passing through live cell membranes, they are also efficiently retained within the cells after loading. As shown in Figure 1, various cell lines incubated with CellMask™ Actin Tracking Stains show no detectable effects on viability after 24 hours of incubation. The stains can detect the F-Actin in live cells (Figures 2-4); paraformaldehyde-fixed cells or tissue, which can be multiplexed with antibody detection (Figures 5-7); and cells used in 3D cell culture for 3D imaging (Figure 11).

Contents and storage

Stain	Catalog No.	Stain Kit Contents	Storage [1]
CellMask™ Green Actin Tracking Stain	A57243	1 vial	Store at 15°C to 30°C. Can be stored to –20°C.
	A57246	5 vials	
CellMask™ Orange Actin Tracking Stain	A57244	1 vial	Store at –20°C to –15°C.
	A57247	5 vials	
CellMask™ Deep Red Actin Tracking Stain	A57245	1 vial	Store at 15°C to 30°C. Can be stored to –20°C.
	A57248	5 vials	
CellMask™ Actin Tracking Stain Variety Pack	A57249	—	Store at –20°C to –15°C.

[1] When stored as instructed, these reagents are effective for a minimum of 6 months after receiving.

Materials required but not supplied

Unless otherwise indicated, all materials are available through **http://www.thermofisher.com**.

- Live specimen, such as cultured or primary cells, 3D cell cultures, spheroids, or organoids
- Plasticware or glassware as needed
- Anhydrous DMSO (Cat. No. D12345)
- Live cell-compatible buffer such as:
  - Live Cell Imaging Solution (Cat. No. A14291DJ)
  - HBSS with calcium and magnesium (Cat. No. 24020117)
  - FluoroBrite™ DMEM (Cat. No. A1896701)
- *(Optional)* NucBlue™ Live ReadyProbes™ Reagent (Cat. No. R37605)
- *(Optional)* Image-iT™ Fixative Solution (4% formaldehyde, methanol-free) or equivalent methanol-free formaldehyde FB002
- (Optional) SYTOX™ Deep Red Nucleic Acid Stain for fixed cells (Cat. No. S11381)
- (Optional) SYTO™ Deep Red Nucleic Acid Stain for live cells (Cat. No. S34901)
- Fluorescent microscope with appropriate filter sets and optics, such as EVOS™ M7000 Imaging System (Cat. No. AMF7000)

Before you begin

- Make a 1000X stock solution of CellMask™ Actin Tracking Stains by dissolving the content of the vial in 60 µL of anhydrous DMSO. This stock solution is stable for at least six months when stored at ≤–20°C.

Live cell staining procedure

1. Dilute the stock solution to 1X in live-cell compatible buffer or cell growth media to make 1X staining solution.
   Note: Lower concentrations can be used in certain cell types.
   Note: Use only staining solution made new on the same day.
2. Apply a sufficient amount of the final staining solution to cover cells adhering to the vessel.
3. Incubate for 30 minutes (1 hour for tissue and 3D spheroids) at 37°C and 5% CO_2.
4. *(Optional)* If needed, nuclear stain can be added in addition to the Actin stains. Add nucleus staining reagent at 1X concentration.
5. Rinse the cells 4 times in a wash buffer (e.g., Live Cell Imaging Solution) at 37°C.
6. Image and analyze cells in buffer.
7. *(Optional)* Fix cells with methanol-free 4% formaldehyde for 15 minutes at room temperature. Cells can be further processed for ICC or IHC using a standard protocol.

Formaldehyde fixation staining procedure

1. Wash the sample 2 times in pre-warmed PBS.
2. Fix the sample in methanol-free 4% formaldehyde solution in PBS for 15 minutes at room temperature.
   Note: Avoid methanol-containing fixatives. Methanol can disrupt actin during the fixation process. We recommend using methanol-free formaldehyde, such as Image-iT™ Fixative Solution (4% formaldehyde, methanol-free) (Cat. No. FB002).
3. Wash the sample 3 times with PBS.
4. *(Optional)* When multiplexing with antibodies, incubate the sample in permeabilization and blocking solution according to standard lab procedure. Perform the primary and secondary antibody incubation according to the manufacturer's protocol.
5. Wash the sample 2 or more times with PBS.
6. Dilute the CellMask™ Actin Tracking Stains stock solution to 1X in PBS or any other compatible buffer to make 1X staining solution. Add sufficient 1X staining solution to cells and incubate for 15 minutes.
7. Wash the sample 3-4 times with PBS.
8. *(Optional)* Nucleus staining reagent can be added at 1X concentration.
9. For long-term storage, mount the sample in a curing aqueous mountant, such as ProLong™ Glass Antifade Mountant (Cat. No. P36980).

Note: Use of methanol, alcohol or any other similar reagent for fixation or permebilization is not recommended.
Note: CellMask™ Actin Tracking Stains are compatible with cryo-preserved, or fresh fixed tissue. These stains are not compatible with FFPE preserved tissue, or any other tissue preparation technique where alcholol or related compound is used.

Figures

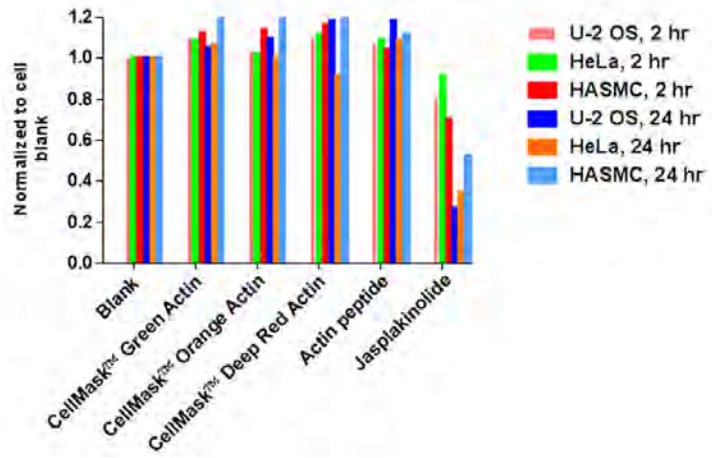

Fig. 1 Cytotoxicity histogram.

HeLa or U2-OS or HASM cells were incubated for 2 hours or 24 hours with 1µM of CellMask™ Green or Orange or Deep Red Actin Tracking Stain. Cytotoxicity was measured using PrestoBlue™ HS Cell Viability Reagent on a Varioskan™ LUX multimode microplate reader. The intensity values were normalized to values of cells without incubation with the CellMask™ Actin Tracking Stains.

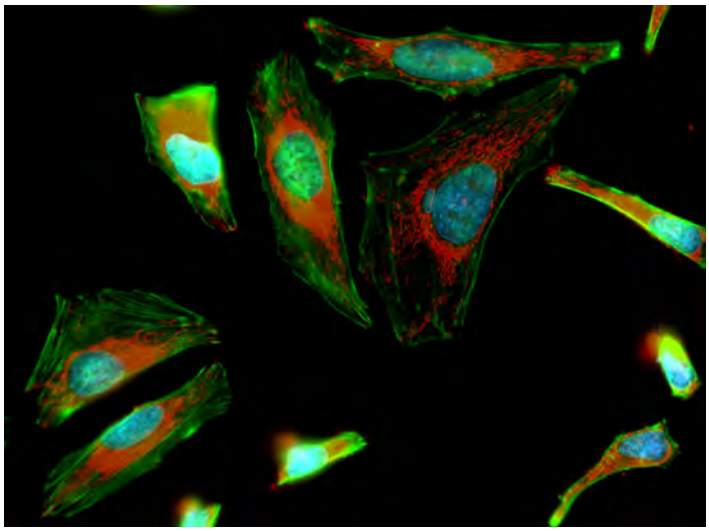

Fig. 2 Live cell labeling with CellMask™ Green Actin Tracking Stain.

Hela cells were grown on a 96-well plate and incubated overnight at 37°C with 5% CO_2. The cells were stained with 1 µM CellMask™ Green Actin Tracking Stain and 500 nM MitoTracker™ Orange stain and Hoechst 34580 for 30 minutes at 37°C. The cells were washed 3 times with HBSS and imaged on an EVOS™ M7000 Imaging System using a 40X objective.

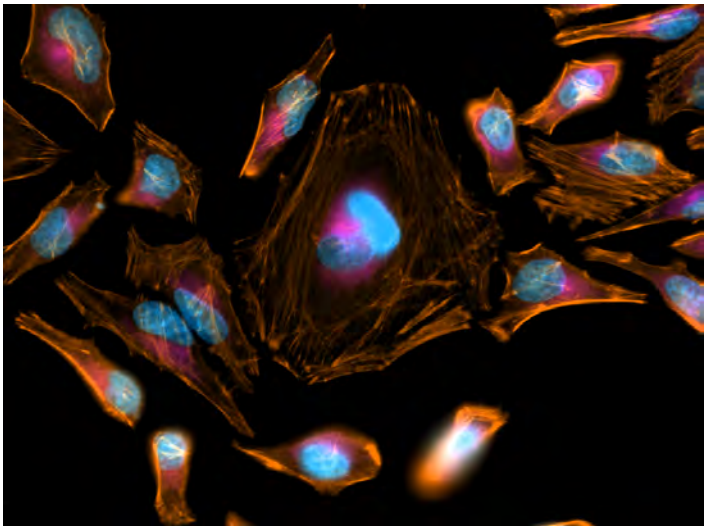

Fig. 3 Live cell labeling with CellMask™ Orange Actin Tracking Stain.

Hela cells were grown on a 96-well plate and incubated overnight at 37°C with 5% CO_2. The cells were stained with CellMask™ Orange Actin Tracking Stain at 1X concentration and 1 µM LysoTracker™ Deep Red stain and Hoechst 34580 for 30 minutes at 37°C. The cells were washed 3 times with HBSS and imaged on an EVOS™ M7000 Imaging System using a 40X objective.

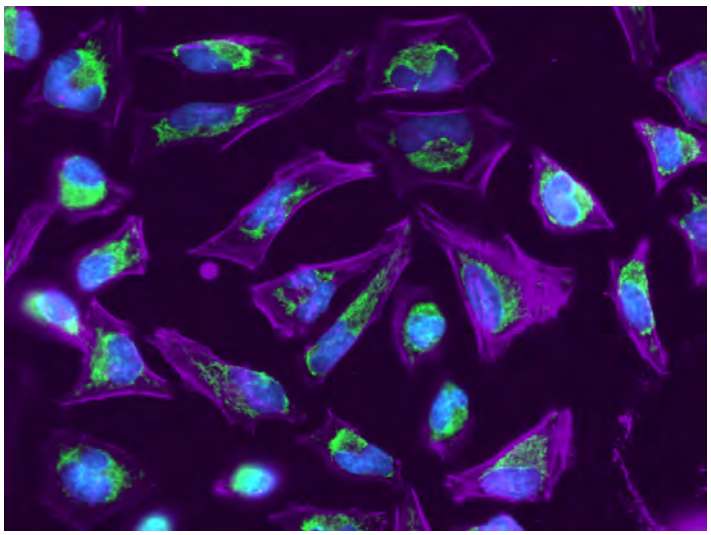

Fig. 4 Live cell labeling with CellMask™ Deep Red Actin Tracking Stain.

Hela cells were grown on a 96-well plate and incubated overnight at 37°C with 5% CO_2. The cells were stained with CellMask™ Deep Red Actin Tracking Stain at 1X concentration and 500 nM MitoTracker™ Green stain and Hoechst 34580 for 30 minutes at 37°C. The cells were washed 3 times with HBSS and imaged on an EVOS™ M7000 Imaging System using a 40X objective.

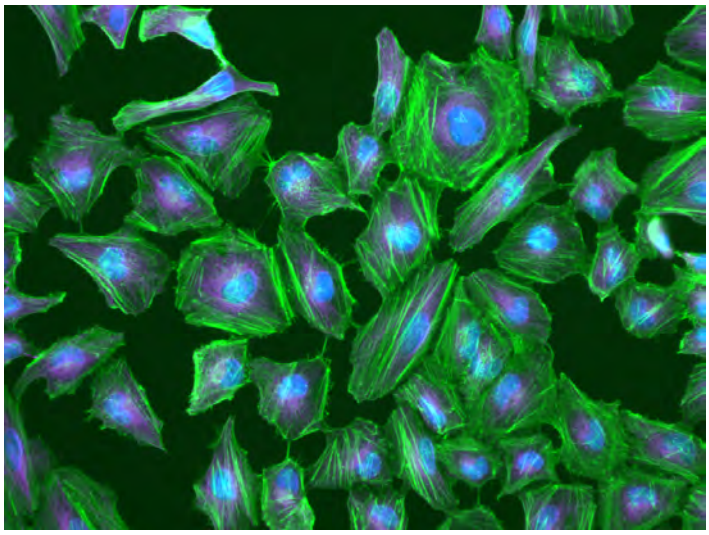

Fig. 5 Fixability of CellMask™ Green Actin Tracking Stain.

Hela cells were grown on a 96-well plate and incubated overnight at 37°C with 5% CO_2. The cells were stained with CellMask™ Green Actin Tracking Stain at 1X concentration for 30 minutes at 37°C. The cells were then formaldehyde-fixed and detergent-permeabilized and stained with a tubulin primary antibody followed by a Goat anti-mouse Alexa Fluor™ Plus 647 secondary antibody and Hoechst 34580 staining. The cells were imaged on an EVOS™ M7000 Imaging System using a 20X objective.

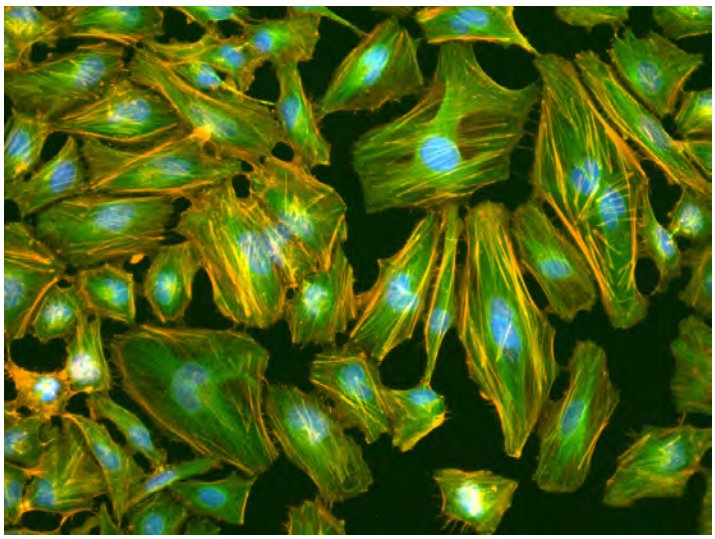

Fig. 6 Fixability of CellMask™ Orange Actin Tracking Stain.

Hela cells were grown on a 96-well plate and incubated overnight at 37°C with 5% CO_2. The cells were stained with CellMask™ Orange Actin Tracking Stain at 1X concentration for 30 minutes at 37°C. The cells were then formaldehyde-fixed and detergent-permeabilized and stained with a tubulin primary antibody followed by a Goat anti-mouse Alexa Fluor™ Plus 488 secondary antibody and Hoechst 34580 staining. The cells were imaged on an EVOS™ M7000 Imaging System using a 20X objective.

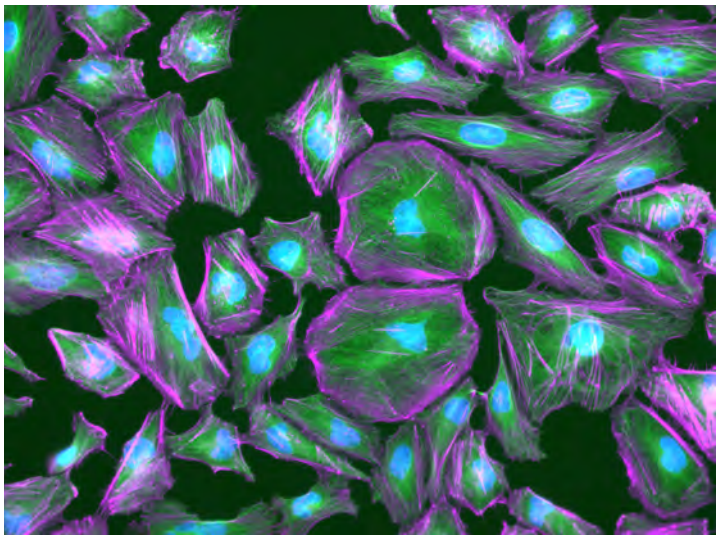

Fig. 7 Fixability of CellMask™ Deep Red Actin Tracking Stain.

Hela cells were grown on a 96-well plate and incubated O/N at 37°C with 5% CO_2. The cells were stained with CellMask™ Deep Red Actin Tracking Stain at 1X concentration for 30 minutes at 37°C. The cells were then formaldehyde-fixed and detergent-permeabilized and stained with a tubulin primary antibody followed by a Goat anti-mouse Alexa Fluor™ Plus 488 secondary antibody and Hoechst 34580 staining. The cells were imaged on an EVOS™ M7000 Imaging System using a 20X objective.

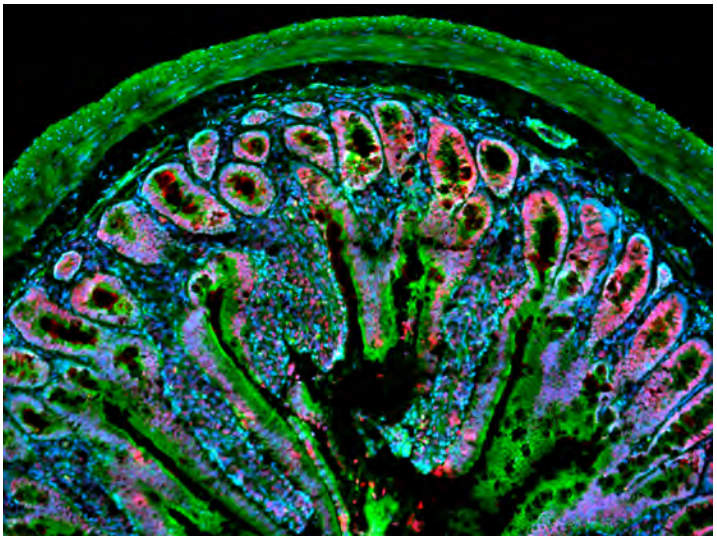

Fig. 8 Labeling of rat duodenal cryo section with CellMask™ Green Actin Tracking Stain.

Rat duodenal cryo section stained with Anti-histone H3 antibody, followed by Goat anti-mouse Alexa Fluor™ Plus 647 secondary antibody following a standard IHC protocol. Tissue sections were further stained with CellMask™ Green Actin Tracking Stain at 1X concentration and Hoechst 34580 for 1 hour and subsequently mounted using ProLong™ Glass Antifade Mountant. The cells were imaged on an EVOS™ M7000 Imaging System.

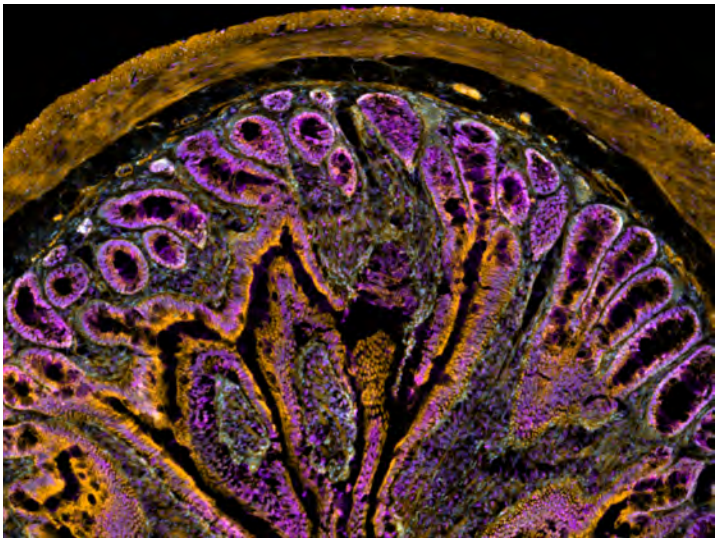

Fig. 9 Labeling of rat duodenal cryo section with CellMask™ Orange Actin Tracking Stain.

Rat duodenal cryo section stained with H3 (96C10) antibody, followed by Goat anti-mouse Alexa Fluor™ Plus 647 secondary antibody following a standard IHC protocol. Tissue sections were further stained with CellMask™ Orange Actin Tracking Stain at 1X concentration and Hoechst 34580 for 1 hour and subsequently mounted using ProLong™ Glass Antifade Mountant. The cells were imaged on an EVOS™ M7000 Imaging System.

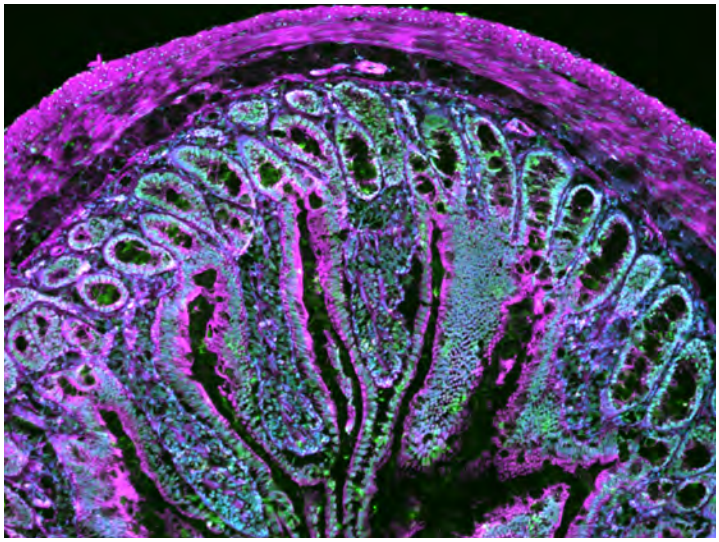

Fig. 10 Labeling of rat duodenal cryo section with CellMask™ Deep Red Actin Tracking Stain.

Rat duodenal cryo section stained with H3 (96C10) antibody, followed by Donkey anti-mouse Alexa Fluor™ Plus 647 secondary antibody following a standard IHC protocol. Tissue sections were further stained with CellMask™ Deep Red Actin Tracking Stain at 1X concentration and Hoechst 34580 for 1 hour and subsequently mounted using ProLong™ Glass Antifade Mountant. The cells were imaged on an EVOS™ M7000 Imaging System.

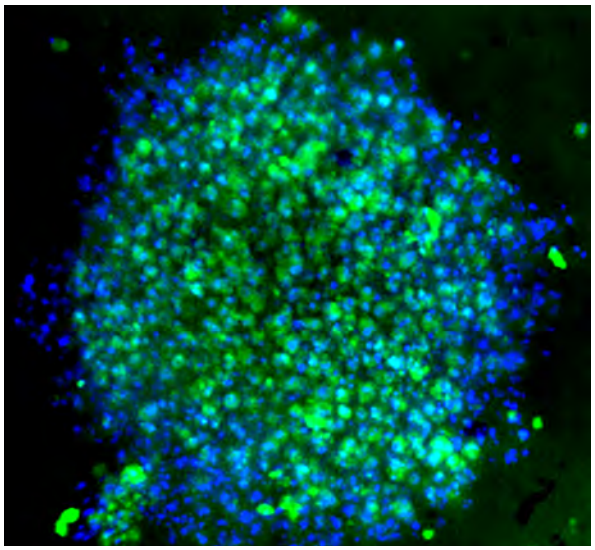

Fig. 11 Labeling of HeLa spheroid with CellMask™ Green Actin Tracking Stain.

HeLa cells plated on a Nunclon™ Sphera™ 96U-well plate at a density of 5K cells/well and left for 24 hours in a CO_2 incubator to form spheroids. Spheroids were stained with CellMask™ Green Actin Tracking Stain at 1X concentration and Hoechst 34580 for 1 hour and then washed 4 times with HBSS. Images were captured at a maximum intensity projection of 50 Z slices at 4 microns each on a CellInsight™ CX7 LZR High Content Analysis Platform.

Limited product warranty

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