HCS CellMask™ Stains

Catalog Numbers H32712, H32713, H32714, H32720, H32721, H32722

Pub. No. MAN0002319 **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

In image-based high-content screening (HCS) assays, cell or object identification is the first step of automated image acquisition and analysis. For many image analysis algorithms, the cell identification process starts with the detection of fluorescently stained nuclei. Using the position of the stained nucleus as a guide, the software then extrapolates to build a mask that marks the probable position of the cytoplasmic region. For some applications, cell identification based on nuclear staining alone is not adequate because the cytoplasmic region assigned by the algorithm does not match that defined by the actual cell boundaries. HCS CellMask™ Stains label the entire cell (cytoplasm and nucleus) for a more thorough description of a cell's anatomy, and provide an accurate backdrop against which the features of interest can be evaluated.

HCS CellMask™ Stains are applied to cells immediately after fixation and permeabilization or after antibody labeling.

Invitrogen™ offers a series of HCS NuclearMask™ reagents for more prominent nuclear labeling in live or fixed cells. For more information on HCS-compatible products, go to **thermofisher.com/HCS**.

Contents and storage

Each HCS CellMask™ Stain provides sufficient material for labeling approximately 10 × 96-well plates using an assay volume of 100 µL.

Contents	Amount	Storage[1]
Component A, one of the following: • Blue stain • Green stain • Orange stain • Red stain • Deep red stain • Near-IR stain	250 µg	• ≤ –20°C • Dessicated • Protected from light
Component B: Dimethylsulfoxide (DMSO)	100 µL	• ≤ 25°C • Dessicated

[1] When stored as directed, product is stable for at least 1 year.

Table 1 Approximate excitation and emission maxima for the HCS CellMask™ Stains

HCS CellMask™ Stain	Cat. No.	Ex/Em (nm)
Blue stain	H32720	346/442
Green stain	H32714	493/516
Orange stain	H32713	556/572
Red stain	H32712	588/612
Deep Red stain	H32721	650/655
Near-IR stain	H32722	777/794

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Flat-bottom 96-well microplates	MLS
Adjustable micropipettors	MLS
Paraformaldehyde 16% aqueous solution for staining fixed cells	MLS
Triton™ X-100	HFH100
PBS, pH 7.4	10010023

General guidelines

- Before using HCS CellMask™ Stains with other fluorophores, perform small-scale optimization studies to ascertain fluorescence compatibility. Depending on the specific experimental conditions and imaging platform specifics, bleedthrough of HCS CellMask™ Stain emission into adjacent channels is often negligible, but can have some impact. Bleedthrough can be mitigated by reducing the final concentration of HCS CellMask™ Stain in the staining solution.
- HCS CellMask™ Stains are extremely bright and can be used in any channel, allowing more flexibility for multiplexing with other fluorophores that are intended to label less abundant targets.
- Select an HCS CellMask™ Stain by matching the dye to the optical characteristics of the detection system.

- For help in selecting the ideal fluorophore for your application or instrumentation, visit our online spectral viewer at **thermofisher.com/spectraviewer**. The spectral viewer can plot the excitation and emission spectra of up to five fluorophores, and can also include excitation and emission filters or laser excitation lines to customize the program to your instrument.

Before first use

1. Allow all vials to warm to room temperature before opening.
2. Prepare a 10 mg/mL HCS CellMask™ Stain stock solution by dissolving the entire contents of the HCS CellMask™ Stain (Component A) in 25 µL of DMSO (Component B).
3. Aliquot, then store HCS CellMask™ Stain stock solution at –20°C, protected from light.

   Note: For optimal results, use frozen aliquots within six months of preparation. Avoid freeze/thaw cycles.

Before each use

- Prepare 4% paraformaldehyde fixative solution by adding 2.5 mL of 16% aqueous paraformaldehyde solution to 7.5 mL of PBS.
- Prepare permeabilization solution by adding 10 µL of Triton™ X-100 to 10 mL of PBS.
- Prepare 1X HCS CellMask™ staining solution by adding 2 µL of HCS CellMask™ stock solution to 10 mL of PBS.

Fix, permeabilize, and stain the cells

1. Remove the medium, then add 4% paraformaldehyde fixative solution to each well.
2. Incubate for 15 minutes at room temperature.
3. Remove the 4% paraformaldehyde fixative solution, then wash the fixed cells 3 times with PBS.
4. Add 0.1% Triton™ X-100 permeabilization solution to each well, then incubate for 15 minutes at room temperature.
5. Remove the permeabilization solution, then wash each well 3 times with PBS.
6. Add 100 µL of HCS CellMask™ staining solution to each well, then incubate for 30 minutes at room temperature.
7. Wash each well 3 times with PBS to remove the excess stain.
8. Seal the plate, then proceed with imaging.

Related products

Product	Cat. No.	Description	Size
HCS NuclearMask™ Deep Red Stain	H10294	250X concentrate in DMSO	400 µL
HCS NuclearMask™ Blue Stain	H10325	for 10 × 96-well plates 2000X concentrate	65 µL
HCS NuclearMask™ Red Stain	H10326	for 10 × 96-well plates 1000X concentrate	125 µL

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision	Date	Description
A.0	14 June 2018	Addition of Near-IR stain and general manual update.