HCS CellMask™ Stains

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
HSC CellMask™ Blue, Green, Orange, Red, or Deep Red stain (Component A)	250 µg	• ≤–20°C • Desiccate • Protect from light	When stored as directed, product is stable for at least 1 year.
Dimethylsulfoxide (DMSO) (Component B)	100 µL	• ≤25°C • Desiccate	
Number of assays: Each HCS CellMask™ product provides sufficient material for approximately 10 × 96-well plates based on the protocol below.			
Approximate fluorescence excitation/emission maxima: See Table 2 and Figure 1.			

Introduction

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 begins 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 (*i.e.*, cytoplasm and the nucleus) for a more thorough description of a cell's anatomy, and provide an accurate backdrop against which the features of interest can be assessed.

HCS CellMask™ reagents are available in a wide range of fluorescent colors from blue to deep red for flexibility in multiparameter analyses (Table 2). When selecting an HCS CellMask™ stain, it is important to not only match the dye to the optical characteristics of the detection system, but to also verify spectral compatibility with the other fluorophores in the experiment. While appropriate instrument configuration and image acquisition conditions are critical for any imaging experiment, target abundance should also be a considered when selecting the optimal segmentation tool. HCS CellMask™ stains are extremely bright and may therefore be used in any channel, allowing more flexibility for multiplexing with other fluorophores intended to label less abundant targets.

HCS CellMask™ stains are applied to cells immediately after fixation and permeabilization or after antibody labeling. Sufficient quantities are provided to stain ten 96-well plates using an assay volume of 100 µL per well. In addition to the HCS CellMask™ stains, Invitrogen also offers a series of HCS NuclearMask™ reagents for more prominent nuclear labeling in live or fixed cells.

Visit www.invitrogen.com/hcs for more information on other HCS-compatible products.

Table 2. Approximate excitation and emission maxima for the HCS CellMask™ stains.

HCS CellMask™ Stain	Ex/Em*
HCS CellMask™ Blue stain	346/442
HCS CellMask™ Green stain	493/516
HCS CellMask™ Orange stain	556/572
HCS CellMask™ Red stain	588/612
HCS CellMask™ Deep Red stain	650/655

*Approximate fluorescence excitation/emission maxima, in nm.

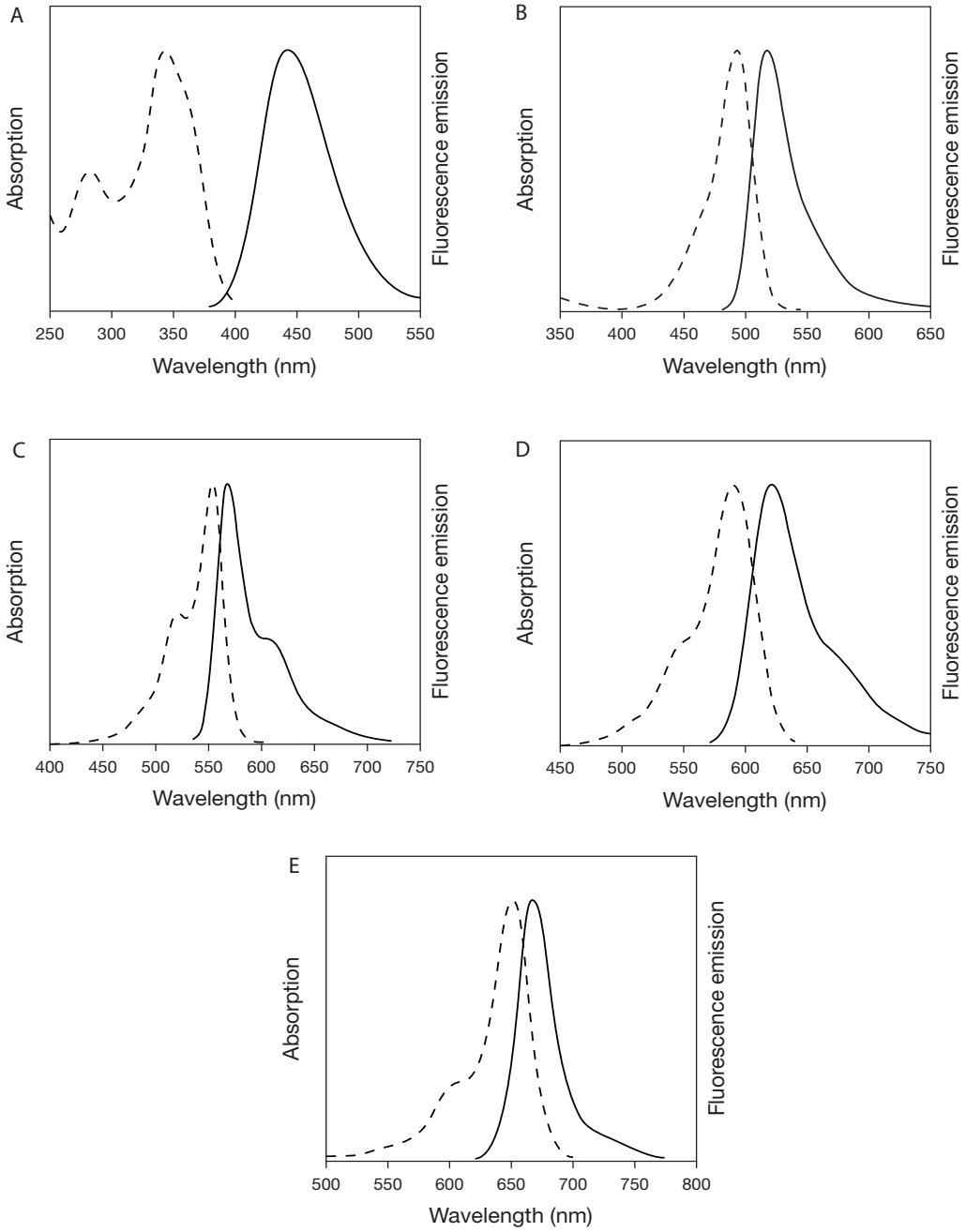

Figure 1. Approximate fluorescence excitation and emission spectra for HCS CellMask™ Blue (panel A), HCS CellMask™ Green (panel B), HCS CellMask™ Orange (panel C), HCS CellMask™ Red (panel D), and HCS CellMask™ Deep Red (panel E) stains.

Before You Begin

Materials Required but Not Provided

- Flat-bottom 96-well microplates
- Paraformaldehyde 16% aqueous solution for staining fixed cells
- Triton® X-100
- Phosphate buffered saline (PBS)

Caution

DMSO is known to facilitate the entry of organic molecules into tissues. Handle reagents containing DMSO using equipment and practices appropriate for the hazards posed by such materials. Dispose of the reagents in compliance with all pertaining local regulations. Always wear protective laboratory clothing and gloves when handling this reagent.

Preparing Stock Solutions

Allow all vials to warm to room temperature before opening.

1.1 Prepare a 10 mg/mL HCS CellMask™ solution by dissolving the entire contents of the HCS CellMask™ stain (Component A) in 25 µL of DMSO (Component B).

1.2 Store any unused HCS CellMask™ stain at –20°C, **protected from light.** For optimal results, use frozen aliquots within six months of preparation. Avoid freeze/thaw cycles.

Prepare the following solutions **fresh** on the day of the assay. The following prepararation provides sufficient material to stain one 96-well plate.

1.3 Prepare the fixative solution by diluting 16% aqueous paraformaldehyde solution with PBS to obtain a 4% paraformaldehyde fixative solution.

1.4 Prepare the permeabilization solution by adding 10 µL of Triton® X-100 to 10 mL of PBS.

1.5 Prepare a 1X HCS CellMask™ staining solution by adding 2 µL of the HCS CellMask™ stock solution (prepared in step 1.1) to 10 mL PBS.

Experimental Protocols

Staining the Cells

2.1 Remove medium and add 4% paraformaldehyde solution (prepared in step 1.3) to each well and incubate for 15 minutes at room temperature.

2.2 Remove fixative and wash the fixed cells 2–3 times with PBS.

2.3 Add the 0.1% Triton® X-100 solution (prepared in step 1.4) to each well and incubate for 15 minutes at room temperature.

2.4 Remove permeabilization solution and wash wells 2–3 times with PBS.

2.5 Add 100 µL of HCS CellMask™ staining solution (prepared in step 1.5) to each well and incubate for 30 minutes at room temperature.

2.6 Wash each well 2–3 times in PBS to remove excess stain before plate sealing and imaging.

Multiplexing with CellMask™ Stains

When using HCS CellMask™ stains with other fluorophores, first perform small-scale optimization to ascertain fluorescence compatibility prior to large-scale screening. Depending upon the specific experimental conditions and imaging platform specifics, bleedthrough of HCS CellMask™ emission into adjacent channels is often negligible, but can have some impact. Given the brightness of these stains, bleedthrough can easily be mitigated by reducing the final concentration of the HCS CellMask™ reagent in the staining solution.

Fluorescence Spectral Characteristics

The fluorescence excitation and emission spectra for the HCS CellMask™ stains are shown in Table 2 and Figure 1.

For additional help in choosing the ideal fluorophore for your application or instrumentation, visit our online spectral viewer at www.invitrogen.com/spectraviewer. You can not only plot the excitation and emission spectra of up to five fluorophores, but also include excitation and emission filters or laser excitation lines to customize the program to your instrument.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
H32712	HCS CellMask™ Red stain *for 10 × 96-well plates*	1 set
H32713	HCS CellMask™ Orange stain *for 10 × 96-well plates*	1 set
H32714	HCS CellMask™ Green stain *for 10 × 96-well plates*	1 set
H32720	HCS CellMask™ Blue stain *for 10 × 96-well plates*	1 set
H32721	HCS CellMask™ Deep Red stain *for 10 × 96-well plates*	1 set
Related Products		
H10294	HCS NuclearMask™ Deep Red stain *250X concentrate in DMSO*	400 µL
H10325	HCS NuclearMask™ Blue stain *for 10 × 96-well plates* *2000X concentrate*	65 µL
H10326	HCS NuclearMask™ Red stain *for 10 × 96-well plates* *1000X concentrate*	125 µL

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