

## HCS NuclearMask™ Stains

**Table 1.** Contents and storage information.

Material	Amount	Concentration	Storage	Stability
HCS NuclearMask™ Blue stain	65 µL	2000X concentrate	<ul style="list-style-type: none"> <li>• 2–6°C</li> <li>• Protect from light</li> </ul>	When stored as directed, this product is stable for at least 1 year.
HCS NuclearMask™ Red stain	125 µL	1000X concentrate in DMSO	<ul style="list-style-type: none"> <li>• ≤–20°C</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	
HCS NuclearMask™ Deep Red stain	400 µL	250X concentrate in DMSO		
<b>Number of assays:</b> Each HCS NuclearMask™ product provides sufficient material for approximately 10 × 96-well plates based on the protocol below.				
<b>Approximate fluorescence excitation/emission maxima:</b> 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.

The versatile HCS NuclearMask™ stains, which survive standard formaldehyde-based fixation and detergent-based permeabilization methods, can be applied to live and fixed cells. HCS NuclearMask™ reagents are available in a wide range of fluorescent colors from blue to deep red for multiplexing flexibility (Table 2). Sufficient quantities are provided to stain ten 96-well plates using an assay volume of 100 µL per well.

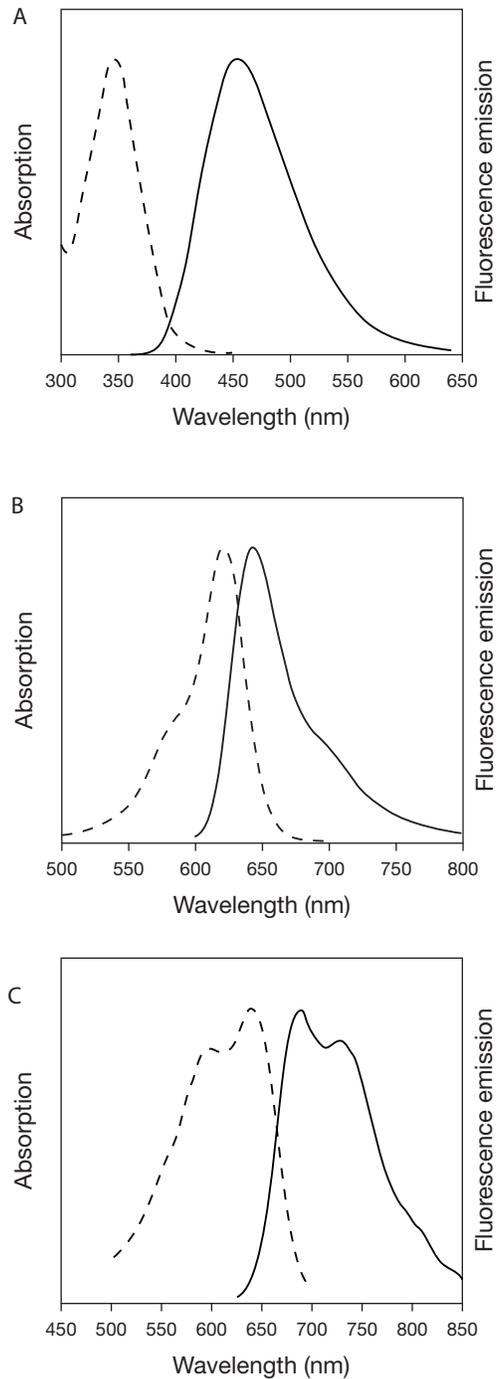
In addition to the HCS NuclearMask™ stains, Invitrogen also offers a series of HCS CellMask™ reagents. In some applications, cell identification based on nuclear staining alone is not adequate because the cytoplasmic region assigned by some image analysis algorithms does not accurately identify the actual cell boundaries. The HCS CellMask™ stains label the entire cell (*i.e.*, cytoplasm and the nucleus), and provide an accurate backdrop against which to assess the features of interest.

Visit [www.invitrogen.com/hcs](http://www.invitrogen.com/hcs) for more information on other HCS-compatible products from Invitrogen.

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

HCS NuclearMask™ Stain	Ex/Em*
HCS NuclearMask™ Blue stain	350/461
HCS NuclearMask™ Red stain	622/645
HCS NuclearMask™ Deep Red stain	638/686

\*Approximate fluorescence excitation/emission maxima, in nm.



**Figure 1.** Fluorescence spectra of HCS NuclearMask™ Blue (panel A), HCS NuclearMask™ Red (panel B), and HCS NuclearMask™ Deep Red (panel C) stains.

## Before You Begin

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### Materials Required but Not Provided

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

### Caution

DMSO (in the reagent) 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

The following protocol prepares sufficient material to stain one 96-well plate using an assay volume of 100  $\mu\text{L}$  per well.

- 1.1 On the day of the assay, prepare a 1X HCS NuclearMask™ staining solution by adding the appropriate amount of stain to 10 mL complete medium (for staining live cells) or appropriate buffer (for staining fixed cells):
  - If using HCS NuclearMask™ Blue stain, add 5  $\mu\text{L}$
  - If using HCS NuclearMask™ Red stain, add 10  $\mu\text{L}$
  - If using HCS NuclearMask™ Deep Red stain, add 40  $\mu\text{L}$
- 1.2 If fixing cells, prepare the fixative solution by diluting 16% aqueous paraformaldehyde solution with PBS to obtain a 4% paraformaldehyde fixative solution.

## Experimental Protocols

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### Staining Live Cells

- 2.1 Remove medium.
- 2.2 Add 100  $\mu\text{L}$  HCS NuclearMask™ cell staining solution (prepared in step 1.1) directly to the cells and incubate for 30 minutes under normal cell culture conditions.
- 2.3 *Optional:* You may fix the cells as described in steps 3.1–3.3.
- 2.4 Proceed to **Imaging and Analysis**.

### Staining Fixed Cells

- 3.1 Remove medium.
- 3.2 Add 4% paraformaldehyde fixative solution (prepared in step 1.2) to each well and incubate for 15 minutes at room temperature.
- 3.3 Remove fixative solution and wash wells once with PBS.

3.4 Add 100  $\mu$ L HCS NuclearMask™ cell staining solution (prepared in step 1.1) directly to the cells and incubate for 30 minutes at room temperature.

3.5 Proceed to **Imaging and Analysis**.

### Imaging and Analysis

4.1 Wash wells once with PBS. You may omit this step; however, washing the wells may improve the signal to noise ratio.

4.2 Add 100  $\mu$ L of PBS to each well and seal the plate.

4.3 Scan the plate using appropriate excitation/emission filters. The fluorescence excitation and emission spectra for the HCS NuclearMask™ stains are shown in Table 2 and Figure 1.

**Note:** For additional help in choosing the ideal fluorophore for your application or instrumentation, visit our online spectral viewer at [www.invitrogen.com/spectraviewer](http://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.

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

## Contact Information

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