HCS Mitotic Index Kit

Catalog no. H10293

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
phospho-H3 rabbit polyclonal antibody (Component A)	25 µL	Not applicable	• ≤–20˚C • Desiccate • Protect from light	When stored as directed this kit is stable for 1 year.
Alexa Fluor® 488 goat anti-rabbit IgG (H+L) *highly cross-adsorbed* (Component B)	25 µL	2 mg/mL		
DAPI (Component C)	20 µL	5 mg/mL aqueous solution		
HCS NuclearMask™ Deep Red stain (Component D)	100 µL	250X concentrate in DMSO		
Number of assays: Sufficient material is supplied for 2 × 96-well plates based on the protocol below.				
Approximate fluorescence excitation/emission maxima: Alexa Fluor® 488 dye: 495/519 nm; DAPI: 358/461 nm, bound to DNA; HCS NuclearMask™ Deep Red stain: 638/686 nm, bound to DNA.				

Introduction

Histones are core proteins of DNA in eukaryotic cells that wrap around the DNA as octamers. The phosphorylation of histone H3 is involved in condensation of chromatin during mitosis and peaks during mitosis. Mitotic H3 phosphorylation occurs at Ser10 of the amino terminus and there is a tight correlation between H3 (Ser10) phosphorylation, chromosome condensation, and segregation during mitosis.[1-3] This event can serve as an indication of mitotic progression or inhibition within the context of drug profiling.

The HCS Mitotic Index Kit allows for the measurement of mitotic cells using automated imaging and analysis, and is amenable to combination with other measurements such as DNA profiling, general cytotoxicity, or immunocytochemical detection of choice targets. The kit includes a primary antibody against phosphorylated histone H3 (Ser10) as a sensitive index of mitosis and a secondary antibody conjugated to the green fluorescent Alexa Fluor® 488 dye. The blue fluorescent DAPI and near infrared fluorescent HCS NuclearMask™ Deep Red stain provide two choices in the kit for DNA profiling and cell demarcation for image analysis. The HCS Mitotic Index Kit represents a powerful image-based assay for the identification of compounds that affect mitotic progression.

As an example, the HCS Mitotic Index Kit was used to detect and quantitate phosphorylated H3 in A549 cells treated with nocodazole (Figures 1 and 2) and validated for robustness and signal change (Tables 2 and 3). While the kit and the following protocol has been validated using cell types such as HeLa and A549 cells, optimization may be needed for other cell types.

The HCS Mitotic Index Kit contains sufficient material to perform the mitotic index assay in two 96-well plates when used as described in the protocol. For larger quantities, inquire at www.invitrogen.com.

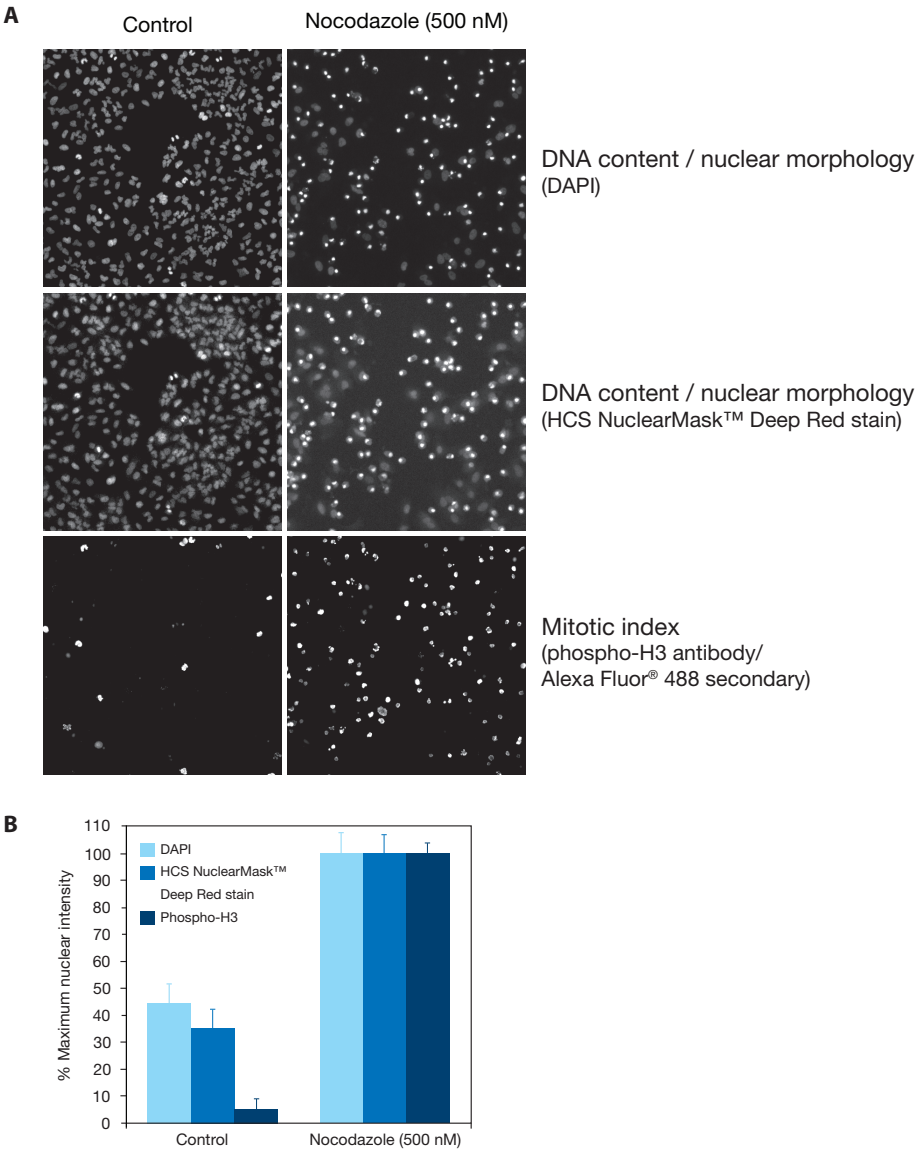

Figure 1. Imaging of mitotic arrest in A549 cells with nocodazole using the HCS Mitotic Index Kit. A549 cells were treated with 500 nM nocodazole for 24 hours at 37°C/5% CO_2 and assayed using the HCS Mitotic Index Kit. Nuclear segmentation and DNA content measurements were done using DAPI or HCS NuclearMask™ Deep Red stain. At 500 nM, there was a strong increase in phospho-H3 staining indicative of mitotic cells. The images were quantitated using the Thermo Scientific Cellomics® ArrayScan® VTI platform (A). The bar graph (B) demonstrates quantitative representation of nocodazole treated cells.

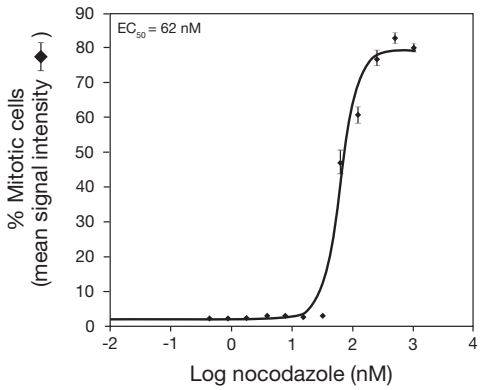

Figure 2. Dose response of nocodazole in A549 cells using the HCS Mitotic Index Kit. A549 cells were treated with nocodazole at final concentrations between 0 to 1,000 nM and incubated for 24 hours. Imaging and analysis was performed using a 10x objective and the Compartmental Analysis BioApplication with the Thermo Scientific Cellomics® ArrayScan® VTI platform. The dose response curve generated by non-linear regression with GraphPad PRISM® was used to determine an EC_{50} value for nocodazole.

Table 2. Assay robustness.*

Analyzed parameter	CV of treated samples (%)
phospho-H3 nuclear intensity	10 ± 1

*A549 cells were treated with 500 nM nocodazole for 24 hours at 37°C/5% CO_2 and the HCS mitotic index assay was performed. Quantitative analysis was performed using the Thermo Scientific Cellomics® ArrayScan® VTI platform and the Compartmental Analysis BioApplication. The data represent %CVs of the averages and standard deviations from treated samples (Max) of three Min/Max plates. CV values were <20% for this parameter.

Table 3. Quantitation of mitototic cells.*

Analyzed parameter	Signal change by treatment (-fold)
phospho-H3 nuclear intensity	20.6 ± 0.5

*A549 cells were treated with 500 nM nocodazole for 24 hours at 37°C/5% CO_2 and mitotic cells were assayed with the HCS Mitotic Index Kit. Quantitative analysis was performed using the Thermo Scientific Cellomics® ArrayScan® VTI and Compartmental Analysis BioApplication. The average intensities and standard deviations were calculated for phospho-H3 nuclear intensity, which measures mitotic cells. The data shown represents the average fold change in signal intensities of treated samples (Max) when compared to the untreated controls (Min) from three Min/Max plates.

Before You Begin

Materials Required but Not Provided

- Phosphate buffered saline (PBS, e.g., D-PBS, Invitrogen Cat. no. 14190-144)
- Cell culture medium
- Paraformaldehyde, 16% aqueous solution
- Flat-bottom 96-well microplates
- Bovine serum albumin (BSA)
- Triton® X-100

Caution

DMSO (in Component D), 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 Cells

Plate cells in appropriate medium the day before adding the test compound. For adherent cells, optimize the cell number and plate coating requirements for the chosen cell model and time span of test compound treatment before performing assay.

Preparing Stock Solutions

Determine whether you will use the blue fluorescent DAPI (Component C) or infrared-fluorescent HCS NuclearMask™ Deep Red stain (Component D) as the nuclear segmentation/DNA content tool with the green fluorescent Alexa Fluor® 488 detection of the phospho-H3 antibody. Only one nuclear stain is required to perform the assay.

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

1.1 Prepare the permeabilization solution by adding 6 µL of Triton® X-100 to 6 mL of PBS.

1.2 Prepare the blocking buffer by dissolving 75 mg of BSA in 25 mL PBS.

1.3 Prepare the primary antibody solution by adding 12 µL of the phospho-H3 antibody (Component A) to 6 mL of blocking buffer (prepared in step 1.2).

1.4 Prepare the secondary antibody/counterstain solution as follows:

- If using DAPI, add 12 µL Alexa Fluor® 488 goat anti-rabbit IgG (Component B) and 5 µL DAPI (Component C) to 6 mL blocking buffer.
- If using HCS NuclearMask™ Deep Red stain (Component D), add 12 µL Alexa Fluor® 488 goat anti-rabbit IgG (Component B) and 40 µL HCS NuclearMask™ Deep Red stain to 6 mL of blocking buffer.

Experimental Protocol

Labeling Cells in 96-well Plates for Imaging

This protocol was developed using A549 and HeLa cells. For other cell types, you may need to modify the protocol. See Figure 3 for the HCS Mitotic Index Kit workflow.

2.1 Add test compound or drug to cells and incubate for the desired period of time under normal cell culture conditions.

The total volume in each well at this step should allow for the eventual addition in step 2.3 of 16% paraformaldehyde fixative directly to the media to prevent loss of mitotic cells.

Note: If using DMSO or any other solvent for dissolving the test compounds, add the same amount of DMSO or other solvent to the control as a vehicle.

2.2 After test compound or drug treatment, **do not** remove the incubation medium from the wells of the 96-well plate.

2.3 Add 16% paraformaldehyde directly to each well for a final concentration of 4% and incubate for 15 minutes at room temperature. It is important to add the fixative directly to the wells to prevent loss of mitotic cells.

2.4 Remove media and wash each well three times with PBS.

2.5 Add 100 µL of permeabilization solution (prepared in step 1.1) to each well and incubate for 15 minutes at room temperature.

2.6 Remove permeabilization solution and wash each well three times with PBS.

2.7 Add 100 µL of blocking buffer (prepared in step 1.2) to each well and incubate for 15 minutes at room temperature.

2.8 Remove blocking solution.

2.9 Add 50 µL of the primary antibody solution (prepared in step 1.3) to each well and incubate for 60 minutes at room temperature.

2.10 Remove primary antibody solution and wash each well three times with PBS.

2.11 Add 50 µL of the secondary antibody/counterstain solution (prepared in step 1.4) to each well

and incubate for 60 minutes at room temperature, **protected from light**.

2.12 Remove secondary antibody/counterstain solution and wash each well three times with PBS.

2.13 Add 100 µL of PBS to each well and proceed to **Imaging and Analysis**.

Imaging and Analysis

Scan the plate using an automated imaging platform equipped with filters appropriate for FITC and DAPI or Alexa Fluor® 647 dye. The nucleus is characterized by the blue fluorescent DAPI or infrared fluorescent HCS NuclearMask™ Deep Red stain. Phospho-H3 staining is quantitated by measuring nuclear intensity in the FITC channel.

When using the Thermo Scientific Cellomics® ArrayScan® VTI platform, use the Compartmental Analysis BioApplication. In channel 1, define the nucleus with DAPI or HCS NuclearMask™ Deep Red stain (the segmentation tool) as objects with Hoechst/XF93 or Cy®5 dye/XF93 filters, respectively. In channel 2, assess the nuclear fluorescence intensity of the phospho-H3 signal using FITC/XF93 filters.

References

1. J Biol Chem 274, 25543 (1999); **2.** Chromosome Res 14, 393 (2006); **3.** Nature 438, 1176 (2005).

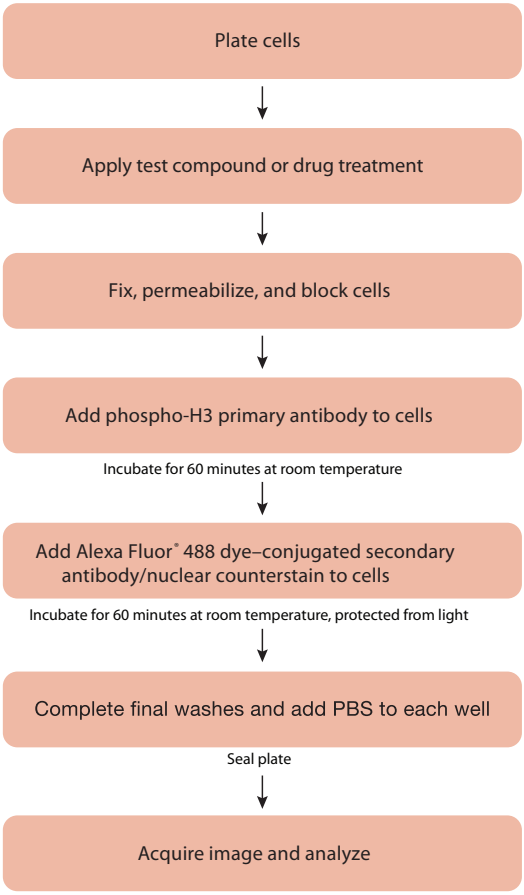

Figure 3. Workflow for the HCS Mitotic Index Kit.

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

Cat. no.	Product Name	Unit Size
H10293	HCS Mitotic Index Kit *2-plate size*	1 kit
Related Products		
C10289	Click-iT® AHA Alexa Fluor® 488 Protein Synthesis HCS Assay *2-plate size*	1 kit
C10045	CellMask™ Orange plasma membrane stain *5 mg/mL solution in DMSO*	100 µL
C10046	CellMask™ Deep Red plasma membrane stain *5 mg/mL solution in DMSO*	100 µL
H10290	HCS LIVE/DEAD® Green Kit *2-plate size*	1 kit
H10292	HCS DNA Damage Kit *2-plate size*	1 kit
H10294	HCS NuclearMask™ Deep Red stain *250X concentrate in DMSO*	400 µL
H10295	HCS Mitochondrial Health Kit *2-plate size*	1 kit
H32711	HCS CellMask™ Red cytoplasmic/nuclear stain *5 mM solution in DMSO* *for high content screening* *for cellular imaging*	125 µL
H34558	HCS CellMask™ Blue cytoplasmic/nuclear stain *for high content screening* *for cellular imaging*	1 set
H34560	HCS CellMask™ Deep Red cytoplasmic/nuclear stain *for high content screening* *for cellular imaging*	1 set
H34157	HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit *for high content screening* *for cellular imaging* *2-plate size*	1 kit
H34158	HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit *for high content screening* *for cellular imaging* *10-plate size*	1 kit
H34350	HCS LipidTOX™ Green phospholipidosis detection reagent *1000X aqueous solution* *for cellular imaging* 10-plate size*	each
H34351	HCS LipidTOX™ Red phospholipidosis detection reagent *1000X aqueous solution* *for cellular imaging* 10-plate size*	each
H34475	HCS LipidTOX™ Green neutral lipid stain *solution in DMSO* *for cellular imaging*	each
H34476	HCS LipidTOX™ Red neutral lipid stain *solution in DMSO* *for cellular imaging*	each
H34477	HCS LipidTOX™ Deep Red neutral lipid stain *solution in DMSO* *for cellular imaging*	each
I10291	Image-iT® DEAD Green™ viability stain *1 mM solution in DMSO*	25 µL

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