



# **BrdU Assay**

**(Mouse primary antibody)**

***For High Content Screening***

For 5 x 96-well plates

**Cat. No. HCS201**

**FOR RESEARCH USE ONLY  
Not for use in diagnostic procedures.**

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## Introduction

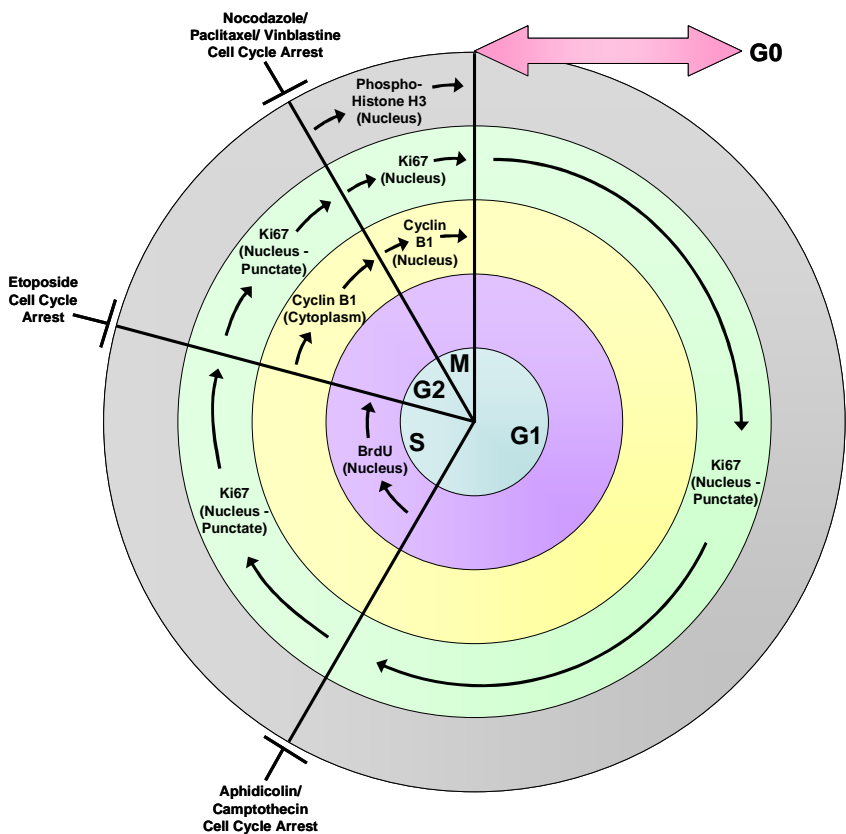
### *(i) High Content Screening and Drug Discovery*

The development of High Content Screening (HCS) technology represents a major step towards improving the drug discovery and development process [1]. HCS enables the evaluation of multiple biochemical and morphological parameters in cellular systems and enables characterization of the subcellular distribution of fluorescent signals with labeled reagents. By combining the automated imaging of cells in microtiter plates with high-quality detection reagents and powerful image analysis algorithms, scientists can now acquire deeper knowledge of multiple morphological or biochemical pathways at the single-cell level, usually in a single assay, at an early stage in the development of new drugs. HCS platforms such as the IN Cell Analyzer (GE Healthcare), ArrayScan (ThermoFisher Scientific), or Opera (Perkin Elmer), can be used to deliver detailed profiles of cellular systemic responses [2].

### *(ii) Principles and Applications of BrdU Detection Assays*

Assessment of the proliferative state of a cell population has become an important parameter in drug discovery research, particularly in evaluating cancer therapeutics and in determining the health of a cell population during ADME/Tox studies. A traditional method for detection of cell proliferation is measurement of [<sup>3</sup>H] thymidine incorporation as cells enter S phase. This technology is slow, labor-intensive and has several limitations, including the handling and disposal of radioisotopes. A well established alternative to [<sup>3</sup>H] thymidine uptake uses bromodeoxyuridine (BrdU), a thymidine analog, to replace [<sup>3</sup>H] thymidine [3, 4]. When cells are pulsed with BrdU, it is incorporated into newly synthesized DNA strands of actively proliferating cells. The incorporation of BrdU into cellular DNA may then be detected using anti-BrdU antibodies, allowing assessment of the population of cells which are synthesizing DNA [3, 4]. This provides an indication of cell proliferation rate and a measurement of the number of cells in S phase during the pulse [5] (see Figure 1).

Cellular processes that influence proliferation are particularly attractive as therapeutic targets, and accordingly, detection of BrdU incorporation has found widespread application in High Content Screening assays [5-10]. These studies have used HCS detection and analysis of BrdU incorporation for target validation, evaluation of genes identified as potential therapeutic or diagnostic targets, and for cell cycle inhibitor profiling, confirming the usefulness of this technique as a tool for drug discovery.



**Figure 1. Eukaryotic cell cycle marker expression and effects of cell cycle phase blocking agents.**

When cells are pulsed with BrdU, it is incorporated into newly synthesized DNA strands of actively proliferating cells. The incorporation of BrdU into cellular DNA may then be detected using anti-BrdU antibodies, allowing assessment of the population of cells which are synthesizing DNA. This provides an indication of cell proliferation rate and a measurement of the number of cells in S phase during the pulse. Compounds such as aphidicolin (an inhibitor of DNA polymerase  $\alpha$ ) and nocodazole (a microtubule depolymerization agent) are capable of arresting the cell cycle at G1/S and G2/M phases, respectively.

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## Application

Millipore's HCS201 BrdU HCS assay provides a complete solution for for the *in vitro* quantitative detection of newly synthesized DNA and quantitation of actively proliferating cells. The reagents in the kit have been specifically optimized for HCS applications.

The assay is designed to enable quantification and characterization of proliferating cells and identification of compounds that inhibit or induce cell proliferation and DNA synthesis via detection of BrdU incorporation. The nuclear dye (Hoechst 33342) may be used for measurements of cell number, DNA content and nuclear size. The kit is suitable for many applications, including cancer drug screening, target validation, cell cycle inhibitor screening, and cytotoxicity assays.

The assay is immunofluorescence-based, and utilizes a high quality mouse monoclonal antibody which identifies BrdU in all species with high sensitivity and specificity. Unlike many anti-BrdU antibodies, this antibody does not require a DNA denaturation step. This is advantageous, as the denaturation process usually involves harsh cell treatment resulting in the loss or inactivation of many cellular proteins, making it difficult to combine additional simultaneous assays and probes for cell function and immunophenotyping. In contrast, this assay may easily be multiplexed with additional probes.

The superior Millipore reagents provided with this kit enable the user to reproducibly generate images with a high signal-to-background ratio, greatly facilitating HCS. In addition, working solutions of the primary and secondary antibodies are stable for at least 24 hours at room temperature (Figure 2), a great benefit for large-scale screening applications. The straightforward sample preparation and processing protocol takes less than 2.5 hrs after fixation.

Reagents are provided for 5 x 96-well microplates – *i.e.*, sufficient to perform 480 separate experiments. The kit includes BrdU reagent, a primary mouse monoclonal antibody for BrdU, a Cy3-conjugated secondary antibody, Hoechst HCS Nuclear Stain, HCS Fixation Solution, HCS Immunofluorescence Buffer, HCS Wash Buffer, and Plate Sealers. Two control compounds, the antibiotic/antiviral agent aphidicolin and the antineoplastic drug nocodazole, along with DMSO for Compound Serial Dilution and Compound Dilution Buffer, are also included in the kit, sufficient for duplicate 12-point dose response samples per plate (see Assay Procedure). Aphidicolin, a DNA polymerase  $\alpha$  inhibitor, and nocodazole, a microtubule depolymerization agent, have both been demonstrated to induce mitotic block, at G1/S phase and G2/M phase, respectively [11-15]. G1/S phase block diminishes the proportion of cells able to newly synthesize DNA (decreased BrdU incorporation), while G2/M phase block has minimal effect on S phase BrdU incorporation (see Figure 1).

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## Kit Components

1. Mouse Anti-BrdU HCS Primary Antibody, 5X: (Part No. CS201677) 1 bottle containing 6 mL.
2. HCS Secondary Antibody (donkey anti-mouse IgG, Cy3 conjugate), 200X: (Part No. CS200437) 1 vial containing 150 µL.
3. Hoechst HCS Nuclear Stain, 200X: (Part No. CS200438) 1 vial containing 150 µL.
4. BrdU, 250X: (Part No. CS201688) 1 vial containing 500 µL.
5. HCS Fixation Solution with Phenol Red, 1X: (Part No. CS201654) 1 bottle containing 100 mL.
6. HCS Immunofluorescence Buffer, 1X: (Part No. CS200435) 1 bottle containing 1000 mL.
7. HCS Wash Buffer, 1X: (Part No. 2007643) 2 bottles containing 500 mL.
8. Aphidicolin (G1/S Arrest Control Compound), 250X: (Part No. CS201656) 1 vial containing 100 µL.
9. Nocodazole (G2/M Arrest Control Compound), 250X: (Part No. CS201689) 1 vial containing 100 µL.
10. DMSO for Compound Serial Dilution: (Part No. CS200441) 1 bottle containing 10 mL.
11. Compound Dilution Buffer: (Part No. CS200442) 1 bottle containing 25 mL.
12. Plate Sealers: (Part No. CS200443) 10 each.

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## Materials Required But Not Provided

1. Sterile, tissue culture-treated black/clear bottom microplates suitable for High-Content Imaging.
2. Cell-type for assay, *e.g.*, HeLa (human cervical adenocarcinoma, ATCC #CCL-2) or A549 (human lung carcinoma, ATCC #CCL-185).
3. Tissue culture instruments/supplies (including 37°C incubator, growth media, flasks/plates, detachment buffer, etc.) for cell type of interest.
4. HCS imaging/analysis system, *e.g.*, GE Healthcare IN Cell Analyzer 1000 with Investigator software. Imaging system must be equipped with beam-splitters and filters capable of reading emission spectra in the blue and red ranges. Example filter ranges are shown in Table 2 below.

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## Warnings and Precautions

1. This product contains hazardous materials. Refer to MSDS for further information.

| <b>Component</b>          | <b>Hazardous Constituent</b>   | <b>Warnings (See MSDS)</b>                                    |
|---------------------------|--------------------------------|---|
| HCS Fixation Solution     | Formaldehyde                   | Toxic, carcinogen, combustible, readily absorbed through skin |
| Hoechst HCS Nuclear Stain | Hoechst 33342                  | Harmful, potential mutagen                                    |
| Aphidicolin               | Aphidicolin                    | Harmful, potential mutagen/carcinogen                         |
| Nocodazole                | Nocodazole                     | Irritant, potential mutagen                                   |
| BrdU                      | BrdU (5-bromo-2'-deoxyuridine) | Harmful, reproductive hazard                                  |
| DMSO                      | Dimethyl sulfoxide             | Combustible, readily absorbed through skin                    |

2. For Research Use Only. Not for use in diagnostic procedures.

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## Stability and Storage

When stored under the conditions indicated on the labels, the kit components are stable up to the expiration date. HCS Fixation Solution, HCS Immunofluorescence Buffer, HCS Wash Buffer, DMSO, Compound Dilution Buffer, and Plate Sealers should be stored at 2-8°C. HCS Primary Antibody, HCS Secondary Antibody, Hoechst HCS Nuclear Stain, BrdU, Aphidicolin and Nocodazole should be stored at -20°C, avoiding repeated freeze/thaw cycles. (Note: If kit is expected to be used for multiple experiments, rather than a single use, thaw antibodies, nuclear stain and control compounds and dispense into appropriately sized aliquots. Store aliquots at -20°C.)

Discard any remaining reagents after the expiration date.

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## Assay Procedure

*Note: The HCS201 assay protocol has been optimized for HeLa human cervical carcinoma (ATCC #CCL-2) and A549 human lung carcinoma (ATCC #CCL-185) cells. However, this kit is suitable for proliferation/cell cycle HCS analysis of a variety of human and alternate species cell types.*

### Cell Preparation:

1. Prior to cell seeding for assay, culture HeLa or A549 cells in growth media until ~70-80% confluent.
2. Detach cells from culture flasks/plates via method appropriate for cell type of interest. Adjust cell density to  $5-7 \times 10^4$  cells/mL in growth media. Add 90  $\mu$ L of this cell suspension (4500-6300 cells) to each well (for a 96-well plate, 4500-6300 cells/well is approximately equivalent to 15,000-21,000 cells/cm<sup>2</sup> of well surface). After adding cells to plate, allow plate to sit on a level surface at room temperature for 15-30 min, which allows for even cell distribution. Following this period, incubate cells in growth media (37°C/5% CO<sub>2</sub>) for 48 hours.
3. Cell treatments (cell-cycle arrest control compounds, test compounds, etc.) can be introduced at any point during this culture period, as appropriate for time-course of treatment of interest. Aphidicolin (G1/S arrest) and nocodazole (G2/M arrest) are provided as cell-cycle arrest control compounds. Sufficient reagents are provided for duplicate 12-point dose response curves (including one DMSO-control set within the dose response) for all five 96-well plates. The compounds are provided at 250X concentration (assuming maximum treatments of 10  $\mu$ M and 25  $\mu$ M for aphidicolin and nocodazole, respectively). Recommended treatment preparation involves half-log ( $1:\sqrt{10}$ ) serial dilution of the 250X compound in DMSO, followed by dilution in Compound Dilution Buffer to 10X. 10 $\mu$ L of each treatment may then be added to the 90  $\mu$ L of culture media already present in each well, for a final 1X concentration (0.4% DMSO). Sample data is provided for 4 hours of compound treatment at 37°C prior to fixation.
4. BrdU can be introduced at any point during this culture period, as appropriate for pulse duration of interest. BrdU is provided at 250X concentration, assuming a 20  $\mu$ M final treatment concentration. Recommended BrdU preparation involves dilution of the 250X compound in Compound Dilution Buffer to 10X. 11  $\mu$ L of 10X BrdU may then be added to the 100  $\mu$ L of culture media/cell treatment already present in each well, for a final 1X concentration. Sample data is provided for 1 hour of 20  $\mu$ M BrdU treatment at 37°C prior to fixation.

## Cell Fixation and Immunofluorescent Staining:

*Note: Staining time is ~2.5 hours post-fixation. Do not allow wells to dry out between staining steps. Aspiration and dispensation of reagents should be conducted at low flow rates to diminish any cell loss due to fluid shear. All recommended 'per well' volumes refer to a single well of a 96-well microplate. All recommended 'per 96-well plate' volumes include 25% excess for liquid handling volume loss. All staining steps are performed at room temperature (RT). All buffers and antibody solutions are stable for at least 24 hours at RT if long-duration automated handling is required (see Figure 1).*

5. At end of culture period, pre-warm HCS Fixation Solution (1X) to room temperature (RT) or 37°C if desired (12 mL/96-well plate). Remove toxin/BrdU-containing culture media and dispose of in compliance with regulations for hazardous waste (see MSDS). Rinse each well twice with 200  $\mu$ L of Wash Buffer. After removing the last Wash Buffer rinse, in a chemical fume hood, add HCS Fixation Solution at 100  $\mu$ L/well and allow to fix for 30 min at RT. Remove fixative and dispose of as hazardous waste. If proceeding immediately to staining, rinse each well twice with 200  $\mu$ L of HCS Immunofluorescence Buffer. Alternatively, if plates are to be stained at a later time, rinse twice with 200  $\mu$ L of Wash Buffer, then leave second rinse volume in wells and store plates tightly sealed at 4°C until staining.
6. If fixed samples have been stored at 4°C prior to staining, rinse twice with 200  $\mu$ L HCS Immunofluorescence Buffer before proceeding with staining protocol.
7. Prepare working solution of Mouse Anti-BrdU HCS Primary Antibody (6 mL/96-well plate) as follows: Add 1.2 mL of thawed primary antibody to 4.8 mL of HCS Immunofluorescence Buffer (see Table 1). Mix well. Remove previous Immunofluorescence Buffer rinse. Add 50  $\mu$ L of primary antibody solution to each well and incubate for 1 hour at RT.
8. Remove primary antibody solution. Rinse three times with 200  $\mu$ L HCS Immunofluorescence Buffer.
9. Prepare working solution of HCS Secondary Antibody/ Hoechst HCS Nuclear Stain (6 mL/96-well plate) as follows: Add 30  $\mu$ L of thawed secondary antibody and 30  $\mu$ L of thawed Hoechst HCS Nuclear Stain to 5.94 mL of HCS Immunofluorescence Buffer (see Table 1). Mix well, protecting solution from light. Remove previous HCS Immunofluorescence Buffer rinse. Add 50  $\mu$ L of HCS Secondary Antibody/Hoechst HCS Nuclear Stain solution and incubate for 1 hour at RT, protected from light.
10. Remove HCS Secondary Antibody/Hoechst HCS Nuclear Stain solution. Rinse twice with 200  $\mu$ L HCS Immunofluorescence Buffer.

11. Remove previous HCS Immunofluorescence Buffer rinse. Rinse twice with 200  $\mu\text{L}$  of HCS Wash Buffer, leaving second rinse volume in wells.
12. Seal plate and image immediately, or store plate at 4°C protected from light until ready for imaging.

| <b>HCS201 Detection Reagent Specifications</b>                       |   |   |  |
|--|---|---|--|
| <i>Primary Antibody working solution</i>                             |   |   |  |
| <b>Reagent</b>   | <b>Required dilution of initial reagent</b> | <b>Vol. required for 1 well (50 <math>\mu\text{L}</math>)</b> | <b>Vol. required for 1 plate (6 mL) (includes ~25% excess)</b> |
| Mouse Anti-BrdU<br>HCS Primary<br>Antibody                           | 1:5   | 10 $\mu\text{L}$  | 1.2 mL   |
| HCS<br>Immunofluorescence<br>Buffer                                  | None  | 40 $\mu\text{L}$  | 4.8 mL   |
| <i>Secondary Antibody/Hoechst HCS Nuclear Stain working solution</i> |   |   |  |
| <b>Reagent</b>   | <b>Required dilution of initial reagent</b> | <b>Vol. required for 1 well (50 <math>\mu\text{L}</math>)</b> | <b>Vol. required for 1 plate (6 mL) (includes ~25% excess)</b> |
| HCS Secondary<br>Antibody  | 1:200                                       | 0.25 $\mu\text{L}$  | 30 $\mu\text{L}$   |
| Hoechst HCS Nuclear<br>Stain   | 1:200                                       | 0.25 $\mu\text{L}$  | 30 $\mu\text{L}$   |
| HCS<br>Immunofluorescence<br>Buffer                                  | None  | 49.5 $\mu\text{L}$  | 5.94 mL<br>(5940 $\mu\text{L}$ )                               |

**Table 1.** Detection Reagent Specifications - HCS201 Mouse Anti-BrdU Assay

## Image Acquisition and Analysis

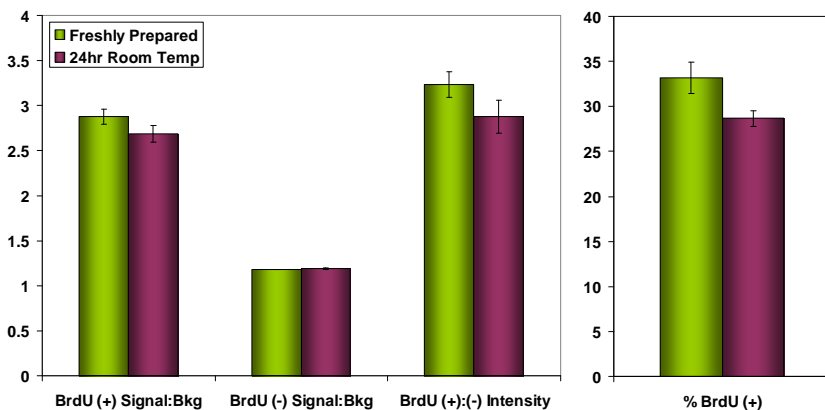
| <b>HCS201 Image Acquisition Guidelines</b>        |                       |  |  |
|---|-----------------------|--|--|
| <b>Detection Reagent</b>                          | <b>Objective Lens</b> | <b>Excitation Filter Range [peak/bandwidth (nm)]</b> | <b>Emission Filter Range [peak/bandwidth (nm)]</b> |
| Hoechst HCS Nuclear Stain                         | 10X                   | 360/40   | 460/40 (or 535/50 if necessary)                    |
| HCS Secondary Antibody, Cy3-donkey anti-mouse IgG | 10X                   | 535/50   | 600/50   |

| <b>HCS201 Image Analysis Guidelines</b> |  |   |   |
|---|--|---|---|
| <b>Cell Parameter</b>                   | <b>Detection</b>                       | <b>Segmentation/ Measurement</b>  | <b>Rationale</b>  |
| Cell Number                             | Hoechst HCS Nuclear Stain              | Nuclear region (460 nm emission channel). Count number of nuclei. DNA content (nuclear intensity) or nuclear area analyses for cell cycle phase are also possible.  | Use total cell number to determine percentage of BrdU-positive cells.                           |
| BrdU Uptake                             | HCS Secondary Antibody, Cy3-conjugated | Nuclear region (600 nm emission channel). Measure Cy3 signal co-localizing with nuclear segmentation. Threshold for positive (+) cells displaying Cy3 nuclear intensity above a user-defined limit. Determine parameters such as percentage/number of (+) or negative (-) cells, etc. | BrdU uptake may be modulated as a result of proliferation rate changes, cell cycle arrest, etc. |

**Table 2.** Image Acquisition and Analysis Guidelines – HCS201 Mouse Anti-BrdU Assay

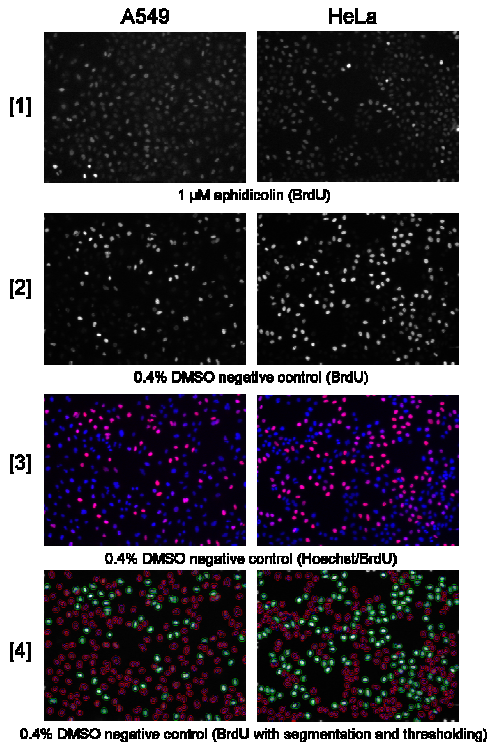
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## Sample Results



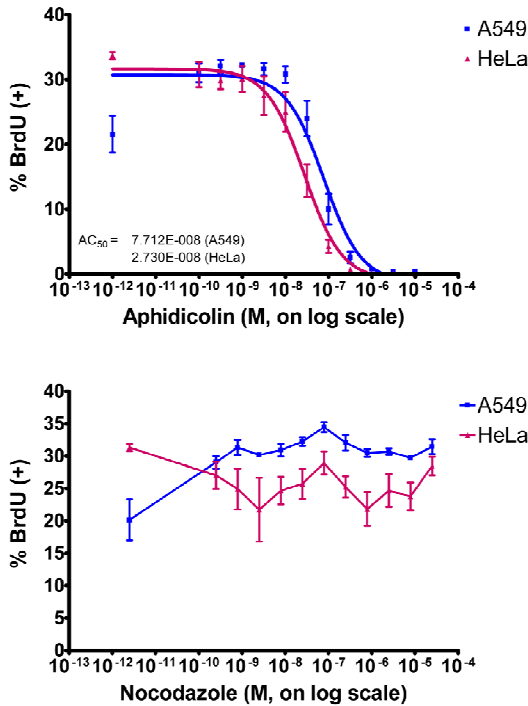
**Figure 2. HCS201 Mouse Anti-BrdU Assay reagent stability.**

A549 cells were seeded at 18,000 cells/cm<sup>2</sup> on 96-well plates in growth media and cultured for 48 hours. 20 $\mu$ M BrdU was introduced for the last hour of culture at 37°C. Samples were fixed and stained under kit conditions, using either fresh buffers and antibody/Hoechst solutions, or buffers and antibody/Hoechst solutions that had been allowed to sit at room temperature (protected from light) for 24 hours prior to staining. Cells were imaged on the GE IN Cell Analyzer 1000 (3.3) at 10X magnification (10 fields/well) and analyzed using the GE IN Cell Analyzer 1000 Workstation (3.4) Multi Target Analysis algorithm. The percentage of BrdU-positive cells was determined by counting the number of Hoechst-segmented nuclei thresholded above an empirically-determined Cy3 nuclear intensity. Signal:background ratios for positive (+) and negative (-) cells were calculated by comparing Cy3 intensities in the nucleus and cytoplasm (as defined by a 3  $\mu$ m wide perimeter surrounding the nucleus). (+):(-) Cy3 nuclear intensity ratios were calculated as a measure of contrast between cells that did or did not incorporate BrdU. Data presented are mean  $\pm$  SD;  $n = 3$ . No significant difference in signal quality was observed between freshly prepared and 24 hour samples.



**Figure 3. Immunofluorescence of untreated and cell cycle-arrested A549 and HeLa cells.**

A549 or HeLa cells were plated at 18,000 cells/cm<sup>2</sup> on 96-well plates in growth media and cultured for 48 hours. Cells were treated for the last 4 hours of culture with either aphidicolin (G1/S arrest) or 0.4% DMSO (negative control). 20 $\mu$ M BrdU was introduced for the last hour of culture at 37°C. Cell handling, fixation and immunostaining were performed according to HCS201 assay protocols. Cells were imaged on the GE IN Cell Analyzer 1000 (3.3) at 10X objective magnification. *Panel [1] and [2]*: Monochromatic images of BrdU incorporation. Note the decrease in BrdU uptake following aphidicolin arrest. *Panel [3]*: Merged images of staining with Hoechst HCS Nuclear Stain (blue) and Mouse Anti-BrdU HCS Primary/Secondary Antibodies (red). *Panel [4]*: Monochromatic images of BrdU fluorescence overlaid with segmentation and thresholding generated by the GE IN Cell Analyzer 1000 (3.4) Workstation Multi Target Analysis algorithm (inner ring = nuclear segmentation, outer ring = 3  $\mu$ m wide nuclear perimeter for cytoplasmic analysis; BrdU (-) cells outlined in red, (+) cells outlined in green).



**Figure 4. Dose response of A549 and HeLa cells to cell-cycle arresting agents.**

A549 or HeLa cells were plated at 18,000 cells/cm<sup>2</sup> on 96-well plates in growth media and cultured for 48 hours. Cells were treated for the last 4 hours of culture with serial dilutions of either aphidicolin (*top panel*, G1/S phase arrest, max. concentration = 10  $\mu$ M) or nocodazole (*bottom panel*, G2/M phase arrest, max. concentration = 25  $\mu$ M). 20 $\mu$ M BrdU was introduced for the last hour of culture at 37°C. Cell handling, fixation and immunostaining were performed as according to HCS201 assay protocols. Cells were imaged on the GE IN Cell Analyzer 1000 (3.3) at 10X (10 fields/well) and analyzed (nuclear segmentation and (+)/(-) intensity thresholding) using the GE IN Cell Analyzer 1000 Workstation (3.4) Multi Target Analysis algorithm. Data presented are mean  $\pm$  SEM,  $n = 4$ . For the G2/M inhibitor nocodazole, minimal dose-dependent effect was observed using the S phase-specific BrdU marker.

## Troubleshooting

| <b><u>Problem</u></b>            | <b><u>Potential Explanations/Solutions</u></b>  |
|----------------------------------|---|
| Weak<br>Cy3/Hoechst<br>signal    | <p>Improper storage or preparation of Primary/Secondary antibody or Nuclear Stain – retry stain with fresh antibody/dye solution.</p> <p>Inadequate primary/secondary antibody or Nuclear Stain concentrations for cell type – titrate dilutions to optimize signal.</p> <p>Signal may diminish in extremely dense cultures – decrease cell seeding concentration or increase primary/secondary antibody or Hoechst concentration.</p> <p>Optimize exposure times and/or fluorescence filters appropriate to fluorophore.</p> |
| Excessive<br>background          | <p>Improper reagent storage or preparation – retry with fresh reagent (antibodies/dyes and/or buffers). Contaminated buffers/solutions may require 0.2µm filter sterilization.</p> <p>Samples may have dried during staining – retry stain on fresh samples.</p> <p>Excessive primary or secondary antibody concentrations for cell type – titrate dilutions to optimize signal.</p> <p>Check for autofluorescence of microplate.</p>   |
| Excessive Cy3/<br>Hoechst signal | <p>Improper preparation of antibody/dye – retry stain with fresh antibody/dye solutions.</p> <p>Inappropriate antibody/dye concentrations for cell type – titrate dilutions to optimize signal.</p> <p>Optimize exposure times and/or fluorescence filters appropriate to fluorophore.</p>  |
| Cell loss                        | <p>Optimize liquid aspiration/dispensation rate to reduce shear.</p> <p>Consider protein-coating to improve cell adhesion to microplate.</p> <p>Optimize cell seeding concentrations for better cell adhesion.</p>  |
| Poor nuclear                     | Effective segmentation parameters can be HCS  |

|   |   |
|---|---|
| <p>segmentation/<br/>intensity<br/>thresholding<br/>during analysis</p>       | <p>system/software-dependent. Consider decreasing cell seeding concentrations for difficulty in analysis of dense cultures (separation of multiple nuclei).</p> <p>Cy3 nuclear intensity thresholding may vary between experiments, depending on cell type, exposure time, duration between staining and imaging, etc. – adjust threshold intensity between (+) and (-) cells for each experiment accordingly.</p>  |
| <p>Excessively<br/>low/high<br/>percentage of<br/>BrdU-positive<br/>cells</p> | <p>Percentages of cells displaying BrdU uptake will vary with cell type, doubling time, seeding density, time in culture, passage number, etc. Optimize culture conditions to maximize expression.</p> <p>The duration and concentration of BrdU utilized in pulse treatment is critical to the amount of BrdU uptake observed. Optimize treatment conditions for experiment-specific parameters of interest (<i>e.g.</i>, a long pulse duration may result in uptake by the majority of the cell population; a low BrdU concentration applied to a high density of cells may result in a lower percentage of BrdU (+) cells/low signal intensity).</p> |
| <p>No dose<br/>response<br/>observed/partial<br/>response curve</p>           | <p>Efficacy of cell cycle arrest control compounds may vary with cell type, cell species, or quality of reagent storage. Use fresh compound, choose alternate maximum/minimum treatment concentrations, or select more appropriate control compounds for cell type of interest.</p> <p>Perform time-course experiments to determine kinetics of compound effects for cell type of interest. Shorter/longer treatment durations may be required.</p>   |

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**Cat No. HCS201**

**01/2010**  
**Revision B, HCS201MAN**