INSTRUCTIONS

Cellomics® BrdU and Ki67 Cell Proliferation Kit

High-Content Screening Reagents

1958.2

Number	Description
8401101	**BrdU and Ki67 Cell Proliferation Kit – Green BrdU and Orange Ki67,** sufficient materials for 1 × 96 wells
8401102	**BrdU and Ki67 Cell Proliferation Kit – Green BrdU and Orange Ki67,** sufficient materials for 5 × 96 wells
8401201	**BrdU Cell Proliferation Kit – Orange BrdU,** sufficient materials for 1 × 96 wells
8401202	**BrdU Cell Proliferation Kit – Orange BrdU,** sufficient materials for 5 × 96 wells
8401301	**Ki67 Cell Proliferation Kit – Orange Ki67,** sufficient materials for 1 × 96 wells
8401302	**Ki67 Cell Proliferation Kit – Orange Ki67,** sufficient materials for 5 × 96 wells

Kit Contents:	**8401101**	**8401102**	**8401201**	**8401202**	**8401301**	**8401302**
BrdU	100 µl	100 µl	100 µl	100 µl	--	--
BrdU Primary Antibody	110 µl	600 µl	110 µl	600 µl	--	--
Ki67 Primary Antibody	8 µl	30 µl	--	--	8 µl	30 µl
DyLight™ Fluor 549 Conjugated Goat Anti-Rabbit IgG	14 µl	72 µl	--	--	14 µl	72 µl
DyLight Fluor 488 Conjugated Goat Anti-Mouse IgG	14 µl	72 µl	--	--	--	--
DyLight Fluor 549 Conjugated Goat Anti-Mouse IgG	--	--	8 µl	72 µl	--	--
DAPI Dye	50 µl	50 µl	50 µl	50 µl	50 µl	50 µl
$MgCl_2$	500 µl	500 µl	500 µl	500 µl	--	--
Wash Buffer (10X Dulbecco's PBS)	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
Permeabilization Buffer (10X Dulbecco's PBS with 1% Triton® X-100)	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml
Blocking Buffer (10X)	85 ml	85 ml	85 ml	85 ml	85 ml	85 ml
Thin Plate Seal Assembly	7/pack	7/pack	7/pack	7/pack	7/pack	7/pack

Storage: Store BrdU Primary Antibody at -20°C. Store all other kit components at 4°C. Keep vials containing the fluorescent antibody and DAPI Dye solutions protected from light. Allow buffers to warm to room temperature before use. See the Solution Preparation section for storage and stability of prepared solutions.

Warning: Please completely read these instructions and the accompanying material safety data sheets before using this product. Cellomics Reagents are not for diagnostic use in humans or animals.

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Introduction

The Thermo Scientific Cellomics BrdU and Ki67 Cell Proliferation Reagent Kits are for simultaneous quantification of DNA replication and Ki67 proliferation marker in the same cell (Figure 1). These kits allow direct measurements of BrdU incorporation and Ki67 expression using a fixed end-point assay based on immunofluorescence detection in cells grown on standard high-density microplates. The primary antibodies are specific for their targets and have minimal cross-reactivity with other targets.

The multiplex kits contain primary antibodies toward BrdU and Ki67 (mouse monoclonal and rabbit polyclonal, respectively) and secondary antibodies conjugated with DyLight Fluor 488 (green) and DyLight Fluor 549 (orange). The orange BrdU singleplex kit contains a mouse monoclonal primary antibody toward BrdU and a DyLight Fluor 549-Conjugated Secondary Antibody. The orange Ki67 probe kit contains a rabbit polyclonal primary antibody toward Ki67 and a DyLight 549-Conjugated Secondary Antibody.

Typical assays that use antibodies to detect DNA-incorporated BrdU require DNA denaturation using harsh treatments such as acid, base or heat. These treatments could result in destruction of cellular morphology, cell integrity and the epitopes of cell surface proteins and other markers. In contrast, HCS Reagent Kits detect BrdU in nuclear DNA without harsh treatments, enabling multiplexed detection of BrdU with antibodies towards other cellular targets.

These kits have been optimized with the Thermo Scientific ArrayScan HCS Reader using the Target Activation BioApplication Software Module but can be used with other Cellomics BioApplications (see the Compatible BioApplication Software Modules Section). Thus, automated plate-handling, focusing, cell image acquisition/processing, and data analysis/management are combined in one high-content screening (HCS) system to assay for test compounds regulating BrdU incorporation and the level of Ki67 nuclear antigen. This assay is sensitive, rapid and easy to use. The evaluation of cell proliferation and other information such as cell number, morphology and analysis of other cellular antigens can be obtained from a single culture. In addition to HCS instruments, cells labeled by the kit reagents can be viewed and analyzed by other fluorescence microscopes.

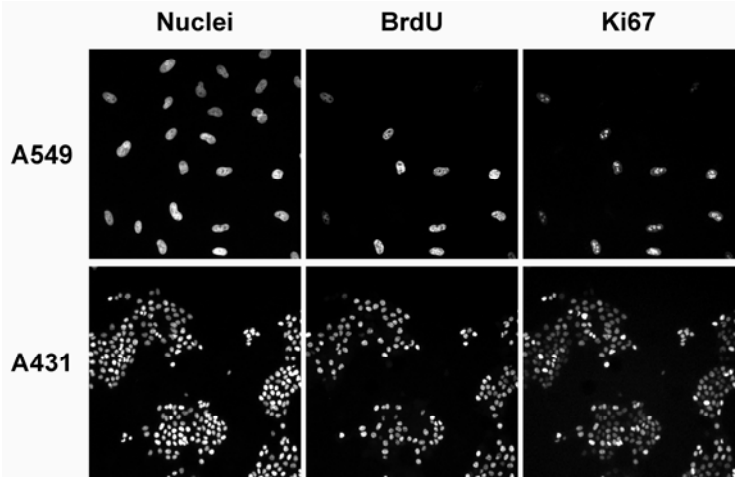

Figure 1. Staining of BrdU and Ki67 in A549 cells (upper panels, 20X objective) and A431 cells (lower pannels, 10X objective). Cells were stained with DAPI (nuclei). Markers were detected according to the kit protocol. The cell images were acquired using a Thermo Scientific ArrayScan HCS Reader. BrdU positive cells are in S phase, Ki67 positive cells are in either G1, S, G2 or M phases and only DAPI positive cells are in G0 phase

Background

Cell proliferation and the characterization of agents that either promote or inhibit cell proliferation are critical areas of cell biology and drug-discovery research. Measuring cell proliferation by the incorporation of 3H-thymidine into cellular DNA is slow and labor-intensive, and its use is limited by the potentially hazardous radioactive materials. As an alternative to 3H-thymidine, 5-bromo-2´-deoxyuridine (BrdU), a thymidine analog, enables detection of DNA replication in actively proliferating cells using a monoclonal antibody directed against BrdU and fluorophore-conjugated secondary antibody. BrdU staining facilitates the identification of cells that have progressed through the S phase of the cell cycle during the BrdU-labeling period. Several protein markers are associated with cell proliferation, including the antigen Ki67, which is expressed during G1, S, G2, and M phases but absent in the G0 phase, such as in quiescent and terminally differentiated cells (Figure 2). Ki67 antigen-positive cells provide a more specific and accurate indication of proliferating cells compared to PCNA-positive cells because PCNA is detectable in almost all quiescent cells.[1,2] Ki67 also correlates with poor survival in ovarian cancer in both univariate and multivariate analyses.[3] The quantification of Ki-67 provides independent prognostic information in breast cancer in addition to tumor size and S-phase fraction.[5]

High-content analysis (HCA) involves a fluorescent cell-based assay in which cells are automatically imaged and analyzed using quantitative fluorescence microscopy. HCA can be used to quantify cell proliferation activity by measuring cell DNA content, the state of cell cycle-associated proteins, and morphological changes in individual cells and in cell populations.[6,7]

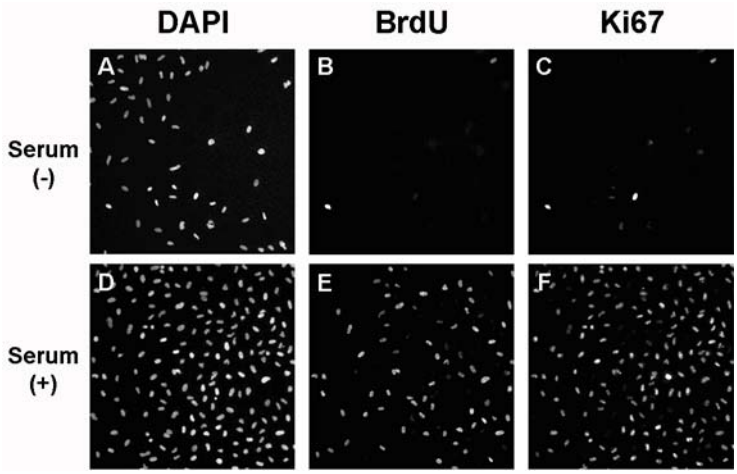

Figure 2. Staining of DAPI (A, D), BrdU (B, E) and Ki67 (C, F) in A549 cells that have been serum-starved for 72 hours, treated with serum-free media only (A, B and C) or with 10% serum for 24 hours (D, E and F). Markers were detected according to the kit protocol. The same cells are shown in panels A-C and in panels D-F. The cell images were acquired using an ArrayScan HCS Reader.

Additional Material Required

- Aphidicolin or other drugs inhibiting S phase
- Nocodazole or other drugs inhibiting G2/M phase
- Paraformaldehyde (37%)
- Clear-bottom 96-well microplates (for example, Biocoat II - Collagen)
- Ultrapure water

Antibody Specificity

Anti-bromodeoxyuridine (BrdU) antibody specifically binds to BrdU and cross-reacts with iododeoxyuridine (10%). Anti-BrdU does not cross-react with fluorodeoxyuridine or any endogenous cellular components, such as thymidine or uridine. Anti-Ki67 antibody specifically detects human and rat Ki67.

Cell Preparation Information

- This protocol is optimized for A549 (American Type Culture Collection # CCL-185) cells; however, the kit is also effective on other cell types including HepG2 and A431 cells. Please see the Cellomics website for more information related to these kits.
- For routine culture of cells, use Minimum Essential Medium-Eagle containing 10% fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin and 100 µg/ml streptomycin, 1X non-essential amino acids solution (= EMEM Complete Medium). For serum starvation study, A549 cells have been thawed and maintained in Dulbeccos Minimum Essential Medium containing high glucose and 10% fetal calf serum, 100 units/ml penicillin and 100 µg/ml streptomycin (= DMEM Complete Medium).
- Dilute cells when they reach 70-80% confluence (two to three times per week) at a dilution of 1:2 to 1:10 depending on cell type. Use cells at a passage number ≤ 20.
- For BrdU detection, with or without Ki67 detection, harvest cells by trypsinization, dilute into EMEM Complete Medium and determine cell density. Dilute cells to 2.5-5 × 10^4 cells/ml in EMEM Complete Medium and add 100 µl per well of a 96-well microplate. Incubate for 16-24 hours at 37°C in 5% CO_2.
- For Ki67 detection with or without BrdU detection, harvest cells by trypsinization, dilute into EMEM Complete Medium and determine cell density. Dilute cells to 3 × 10^4 cells/ml and to 3 × 10^5 cells/ml in EMEM Complete Medium. Add 100 µl of the 3 × 10^4 cells/ml to half of a Biocoat II - Collagen 96-well microplate and add 100 µl of the 3 × 10^5 cells/ml to the other half. Incubate for 16-24 hours at 37°C in 5% CO_2 (= 3,000 cells/well and 30,000 cells/well).

Procedural Notes

- DNA content (2N vs. 4N) is determined by nocodazole treatment and DAPI staining. S phase analysis is achieved by aphidicolin treatment and BrdU incorporation. Cell density-dependent contact inhibition is determined by Ki67 expression.
- Do not allow plate wells to become dry at any time during the protocol.
- Perform all steps at room temperature unless otherwise indicated.
- The protocol given below requires approximately 3 hours post-compound treatment to complete.
- This protocol is optimized for A549 cells cultured in 96-well plates. Using conditions other than those indicated may necessitate optimization.
- Please refer to the Compatible BioApplication Software Modules Section for Cellomics applications that can be used with this kit and the ArrayScan HCS Protocol instructions for optimal assay implementation on the ArrayScan HCS Reader.
- DyLight Fluor 488 Conjugates have approximate absorption/emission maxima of 494/532 nm. DyLight 549 Conjugates have approximate absorption/emission maxima of 562/572 nm. DAPI has an approximate absorption/emission maxima of 358/461 nm.

- The total intensity from a DAPI-labeled nucleus, determined on an image analysis system such as on Cellomics ArrayScan HCS Reader, is proportional to the nucleus' DNA content. DAPI staining can be used to determine cell-cycle phase only within the linear range of the dye, which can vary depending on cell type. Incubating BrdU primary antibodies longer than one hour at 37°C may diminish DAPI staining intensity.
- Cells prepared and labeled according to these instructions can be analyzed by fluorescence microscopes using the appropriate filter set(s) or confocal microscopy. Optimization may be required when using slides, coverslips or multi-well chamber slides. Use image-processing software to quantify the targets.

Solution Preparation (per 96-well plate)

1X Wash Buffer	Add 20 ml 10X Wash Buffer to 180 ml ultrapure water for a final volume of 200 ml. Store buffer at 4°C for up to 7 days.
Fixation Solution	Add 2 ml of 37% formaldehyde solution to 18 ml of 1X Wash Buffer and heat to 37°C in a water bath just before use. Pre-warming the Fixation Solution is critical to maintaining cell integrity. Prepare solution just before each assay.
1X Permeabilization Buffer	Add 2 ml of 10X Permeabilization Buffer to 18 ml of the 1X Wash Buffer. Store buffer at 4°C for up to 7 days.
1X Blocking Buffer	Add 10 ml of the 10X Blocking Buffer to 90 ml of 1X Wash Buffer to make 100 ml of 1X Blocking Buffer. Store buffer at 4°C for up to 7 days.
Primary Antibody Solution	For the BrdU and Ki67 multiplex kit, add 60 µl of $MgCl_2$, 100 µl of BrdU Primary Antibody and 6 µl of Ki67 Primary Antibody to 5.9 ml of 1X Blocking Buffer. Prepare solution just before each assay. For the BrdU singleplex kit, add 60 µl of $MgCl_2$, 100 µl of BrdU Primary Antibody to 5.9 ml of the 1X Blocking Buffer. Prepare solution just before each assay. For the Ki67 singleplex kit, add 6 µl of the Ki67 Primary Antibody to 6 ml of the 1X Blocking Buffer. Prepare solution just before each assay.
Staining Solution (Secondary Antibody)	For the BrdU and Ki67 multiplex kit, add 3 µl of DAPI, 6 µl of the DyLight 488 Goat Anti-Mouse and 12 µl of the DyLight 549 Goat Anti-Rabbit to 6 ml of 1X Blocking Buffer. Prepare solution just before each assay. For the BrdU singleplex kit, add 3 µl of DAPI and 6 µl of the DyLight 549 Goat Anti-Mouse to 6 ml of 1X Blocking Buffer. Prepare solution just before each assay. For the Ki67 singleplex kit, add 3 µl of DAPI and 12 µl of the DyLight 549 Goat Anti-Rabbit to 6 ml of 1X Blocking Buffer. Prepare solution just before each assay.

Procedure for BrdU Incorporation Analysis – Aphidicolin Treatment

Note: Ki67 level is unaffected by aphidicolin.

1. From the aphidicolin stock solution (e.g., 1 mg/ml in DMSO), dilute aphidicolin to 3 µg/ml, or other test compound to appropriate concentration, in culture medium and add 50 µl to each treatment well. Add 50 µl of culture medium to the control wells. Incubate plate for 1 hour at 37°C in 5% CO_2.
2. Dilute the BrdU (100 mM) to 160 µM in warm culture medium (37°C) (e.g., 12 µl BrdU to 7.5 ml medium for one plate) and add 50 µl to each control well and treatment well. Incubate plate for 30 minutes at 37°C in 5% CO_2.
3. Aspirate culture medium and add 100 µl of warmed Fixation Solution to each well. Incubate plate in a fume hood at room temperature for 15 minutes.

   Note: Using warm (37°C) Fixation Solution is critical for maintaining cell integrity.
4. Aspirate Fixation Solution and wash plate twice with 100 µl/well of 1X Wash Buffer.
5. Aspirate buffer, add 100 µl/well of 1X Permeabilization Buffer and incubate for 15 minutes at room temperature.
6. Aspirate Permeabilization Buffer, add 100 µl/well of 1X Blocking Buffer and incubate at room temperature for 15 minutes.

7. Aspirate Blocking Buffer and add 50 µl/well of Primary Antibody Solution. Incubate plate for 1 hour at 37°C.

   Note: To reduce variation between wells when using multiple plates, spread plates in the incubator (i.e., do not stack plates). Room temperature incubation will diminish signal intensity.
8. Aspirate Primary Antibody Solution and wash plate twice with 100 µl/well of 1X Blocking Buffer.
9. Aspirate buffer and add 50 µl/well of Staining Solution (Secondary Antibody). Incubate plate for 30 minutes at room temperature protected from light.
10. Aspirate Staining Solution and wash plate twice with 100 µl/well of 1X Wash Buffer.
11. Aspirate buffer and replace with 150 µl/well of 1X Wash Buffer.
12. Seal plate and evaluate on the ArrayScan HCS Reader. Store plates at 4°C.

Procedure for Contact Inhibition Analysis

Note: This protocol is optimized for A549 cells but is effective with other cell lines including primary cells. For best results, optimize the cell density for each cell line.

1. Plate A549 cells in the control wells with 3,000 cells/well and 30,000 cells/well in the test wells. Incubate for 24 hours at 37°C in 5% CO_2.
2. For the BrdU kit (if using the Ki67 kit, skip this step) dilute the BrdU (100 mM) to 120 µM in warm (37°C) culture medium (for example, 9 µl BrdU in 7.5 ml medium for one plate) and add 50 µl to each control and test well. Incubate plate for 30 minutes at 37°C in 5% CO_2.
3. Aspirate culture medium and add 100 µl/well of warmed Fixation Solution. Incubate plate in a fume hood at room temperature for 15 minutes.

   Note: Using warm (37°C) Fixation Solution is critical for maintaining cell integrity.
4. Aspirate Fixation Solution and wash plate twice with 100 µl/well of 1X Wash Buffer.
5. Aspirate buffer, add 100 µl/well of 1X Permeabilization Buffer and incubate for 15 minutes at room temperature.
6. Aspirate Permeabilization Buffer, add 100 µl/well of 1X Blocking Buffer and incubate at room temperature for 15 minutes.
7. Aspirate Blocking Buffer. To detect BrdU, add 50 µl/well of BrdU Primary Antibody Solution and incubate for one hour at 37°C. To detect Ki67, add 50 µl/well of Ki67 Primary Antibody Solution and incubate for one hour at room temperature.

   Note: For BrdU detection using multiple plates, minimize variation between wells by spreading plates in the incubator (i.e., do not stack plates). Room temperature incubation will diminish signal intensity.
8. Aspirate Primary Antibody Solution and wash twice with 100 µl/well of 1X Blocking Buffer.
9. Aspirate Blocking Buffer and add 50 µl/well of Staining Solution (Secondary Antibody). Incubate for 30 minutes protected from light at room temperature.
10. Aspirate Staining Solution and wash plate twice with 100 µl/well of 1X Wash Buffer.
11. Aspirate Wash Buffer and replace with 150 µl/well of 1X Wash Buffer.
12. Seal plate and evaluate on the ArrayScan HCS Reader. Store plate at 4°C.

Procedure for DNA Content Analysis – Nocodazole Treatment

1. Dilute the nocodazole stock solution (e.g., 1 mg/ml in DMSO) to 1.5 µg/ml (or other test compounds to appropriate concentration) in culture medium and add 50 µl to each treatment well. Add 50 µl of culture medium to the control wells. Incubate for 24 hours at 37°C in 5% CO_2.
2. Add 60 µl/well of warmed Fixation Solution on top of culture media. Incubate plate in a fume hood at room temperature for 30 minutes.

   Note: Using warm (37°C) Fixation Solution is critical to maintaining cell integrity.

3. Aspirate Fixation Solution and wash plate twice with 100 µl/well of 1X Wash Buffer.
4. Aspirate Wash Buffer, add 100 µl/well of 1X Permeabilization Buffer and incubate for 15 minutes at room temperature.
5. Aspirate Permeabilization Buffer, and then add 50 µl/well of DAPI Staining Solution (3 µl of DAPI in 6 ml 1X Wash Buffer). Incubate for 15 minutes at room temperature protected from light.
6. Aspirate Staining Solution and then replace with 150 µl/well of 1X Wash Buffer.
7. Seal plate and evaluate on the ArrayScan HCS Reader. Store plates at 4°C.

Application Data

Note: A549 cells have been tested for the cell proliferation assay from passage 2 to 20.

A. Nocodazole Treatment and DNA Content Analysis

Nocodazole treatment blocks the G2/M cell cycle phase and increases the number of cells with 4N DNA content. The majority of the non-treated control cells have 2N DNA content (Figure 3).

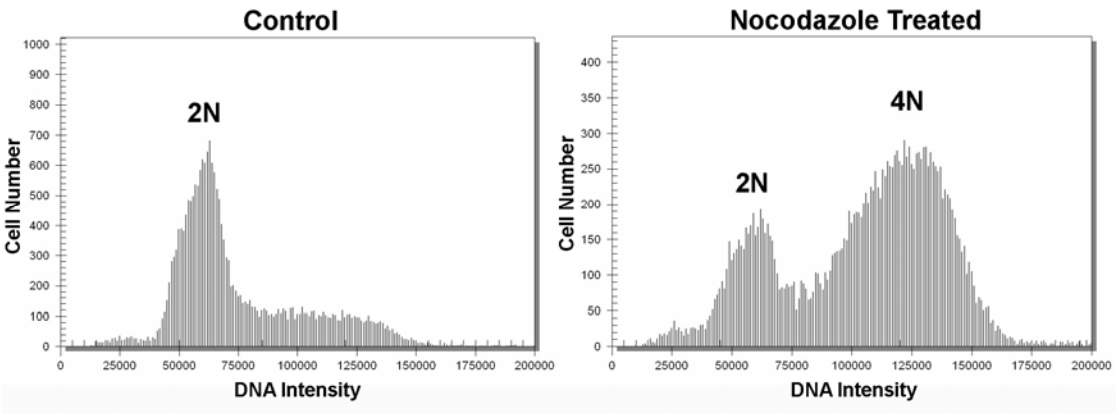

Figure 3. Nuclear intensity of A549 cells measured with DAPI staining. A549 cells were treated with nocodazole (0.5 µg/ml) for 18 hours and then fixed, permeabilized and stained with DAPI.

The robustness of DNA content analysis was ascertained by determining the Z´ factor for the total nuclear DNA content (Peak Nuclear Total Intensity) of non-treated (min, 0 ng/ml) and nocodazole-treated (max, 0.5 µg/ml for 18 hours) wells. Z´ value (DNA content analysis): 0.601 ± 0.030.

B. Cell Contact Inhibition

Contact inhibition in high density cell culture suppresses BrdU incorporation and Ki67 expression in A549 cells compared to the normal cell density culture (Figure 4).

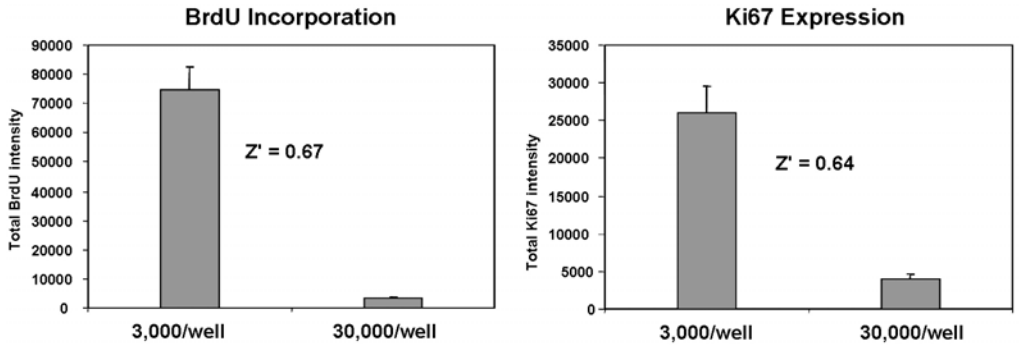

Figure 4. High density cell culture suppresses BrdU incorporation and Ki67 expression. A549 cells were plated with 30,000 cells per well and incubated for 24 hours. The control wells had normal density of 3,000 cells per well.

For the BrdU and Ki67 kit, Z´ value was determined for total intensity in the nucleus of control wells (max, cell density of 3,000 cells/well) and test wells (contact inhibited, cell density of 30,000 cells/well). The mean ± SD of the Z´ was determined from three plates of A549 cells that were incubated identically. The Z´ values are as follows:

BrdU: 0.675 ± 0.050

Ki67: 0.643 ± 0.040

C. Serum Stimulation Response Curves

BrdU incorporation and Ki67 activation in A549 cells were measured simultaneously in the same cells as described in the procedure. The feature plotted is the difference in normalized total intensity in the nuclear regions for each target (Figure 5). Levels of both BrdU and Ki67 increase initially after serum stimulation, which indicates cell proliferation is activated.

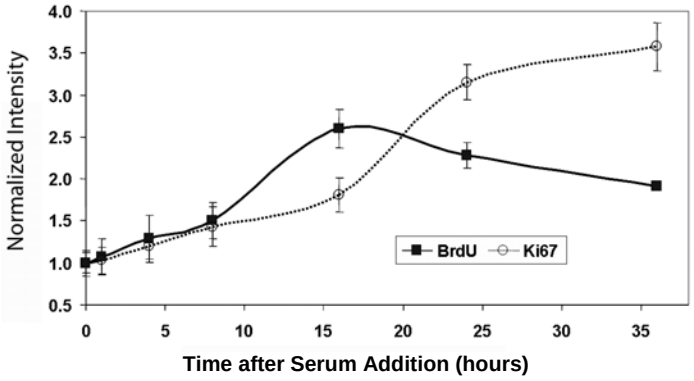

Figure 5. Response to serum stimulation. A549 cells were maintained in DMEM complete media before serum starvation. After 72 hours of serum starvation, serum (10% final concentration) was added to the cell culture medium. Data represents mean ± SD from three plates (eight wells per 96-well plate per time point).

Note: For the serum starvation experiment, the cell cycle time is influenced by cell density, cell passage number and culture condition. For best results determine the appropriate cell density and cell passage in addition to the time for serum starvation and stimulation. (Non-cancer cells respond more consistently to serum depletion and stimulation than the cancer cell lines.)

D. Cell Cycle Inhibitory Drugs

As determined by the ArrayScan Instrument, aphidicolin treatment inhibits BrdU incorporation in the cell and blocks S phase in the cell cycle (Figure 6).

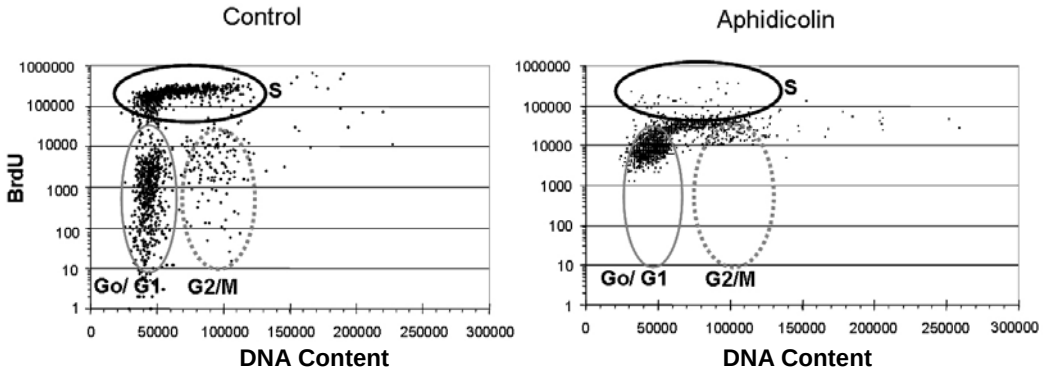

Figure 6. Cell population analysis of A549 cells treated with aphidicolin. BrdU and DNA content measurement separates cell cycle phases. G0/G1, G2/M, S: cell cycle phases.

The robustness of BrdU detection was ascertained by determining the Z´ for the total BrdU intensity in the nucleus of non-treated (min, 0 ng/ml) and aphidicolin-treated (max, 1 µg/ml for an hour) wells. The mean ± SD of the Z´ was determined from three plates of A549 cells that were treated identically. The Z´ value for BrdU was 0.465 ± 0.062.

The S phase inhibiting drugs (hydroxyurea and aphidicolin) inhibited BrdU incorporation; other drugs (e.g., cycloheximide and mimosine) also inhibited BrdU incorporation (Figure 7).

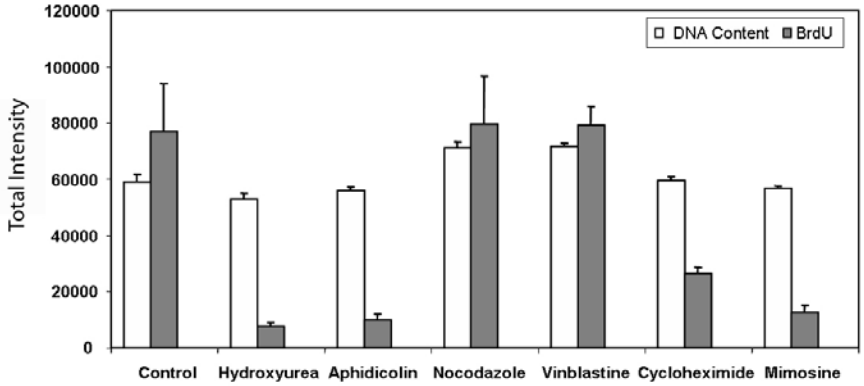

Figure 7. Drugs inhibited BrdU incorporation. A549 cells were treated with drugs (10 mM hydroxyurea; 1 µg/ml aphidicolin; 0.5 µg/ml nocodazole; 0.3 µM vinblastine; 10 µg/ml cycloheximide; 0.5 mM mimosine) for 4 hours and BrdU incorporation was measured.

E. Dose Response Curves for BrdU

The BrdU incorporation in A549 cells was measured after 2 hours treatment of each drug. The feature plotted is the difference in BrdU incorporation in the nuclear regions (Figure 8). The IC_{50} concentrations for the inhibition of BrdU are indicated.

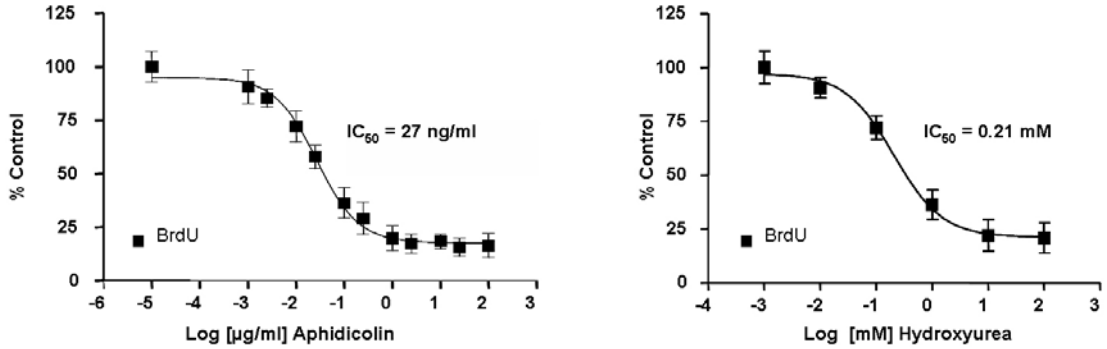

Figure 8. Incorporation of BrdU in response to treatments. Data represents mean ± SD from three plates (eight wells per 96-well plate per time point).

Recommendations for Automation

- Plating Cells: To improve the uniformity and throughput of plating cells, use a liquid handling system such as Thermo Scientific Multidrop Combi or WellMate Dispensers.
- Dead Volumes: Every piece of automation instrumentation has a non-recoverable dead volume associated with it. Be aware of these dead volumes, priming volumes and rinsing volumes when calculating your reagent requirements.
- Nonspecific Binding: Because of the potential of reagent interaction with large surface areas inherent to tubing, syringes and peristaltic pumps, pre-priming with reagents or pre-coating with protein blockers may be warranted.
- Mixing: Gentle mixing may be required when adding a DMSO-based solution to keep overly concentrated solutions from lying on top of the cell layer. Be careful not to dislodge cells or beads during mixing procedures.
- Cell Washing: Use an automated plate washer designed to gently wash attached cells. Be careful not to dislodge cells or beads during cell washing.
- Incubation: Minimize the time when plates with live cells are out of a controlled CO_2 environment. For best results, use an automated incubator to deliver plates to a pipetting deck.

- Exposure: Minimize operator exposure to fixative by some form of containment. Some reagents and compounds are light-sensitive; be aware of these constraints when scaling up for an automated run.
- Adapting to other plate formats: When using different plate types, adjust reagent volumes as needed. Some suggested starting volumes are listed in Table 1.

Table 1. Suggested volumes to use for different cell culture plates.

Kit Component	96-Well Plates (µl/well)	384-Well Plates (µl/well)	24-Well Plates (µl/well)
Fixation Solution	100	25	400
1X Wash Buffer	100	25	400
Wash Buffer II	100	25	400
1X Permeabilization Buffer	100	25	400
1X Blocking Buffer	100	25	400
Antibody Solution	50	12.5	200
Staining Solution	50	12.5	200
1X Wash Buffer (final wash)	150	37.5	200

Compatible BioApplication Software Modules

S50-0021-2 or S50-2021-1 **Cell Cycle BioApplication**

S50-0017-1 or S50-2017-1 **Compartmental Analysis BioApplication**

S50-0011-1 or S50-2011-1 **Target Activation BioApplication**

References

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Thermo Scientific Cellomics HCS Reagent Kits are developed and manufactured at the same Thermo Fisher Scientific Inc. facility as Pierce Protein Research Products and are supported by Pierce Technical Support (see contact information in page footer). These kits are part of the Cellomics Total Solution Platform for HCS, which also includes Cellomics ArrayScan and other HCS Instrumentation, BioApplication Image Analysis Software and High-Content Informatics. For more information, visit *www.thermo.com/cellomics* or call 800-432-4091 (toll free) or 412-770-2500.