# ab112116

# Cell Cycle Assay Kit – Green Fluorometric

## Instructions for Use

For monitoring cell cycle progression and proliferation in live cells using our proprietary Nuclear Green CCS1

This product is for research use only and is not intended for diagnostic use.

Version 5b Last Updated 29 October 2018

# Table of Contents



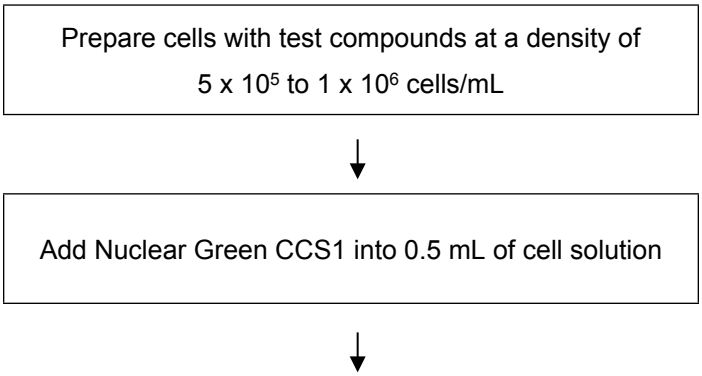
# 1. Introduction

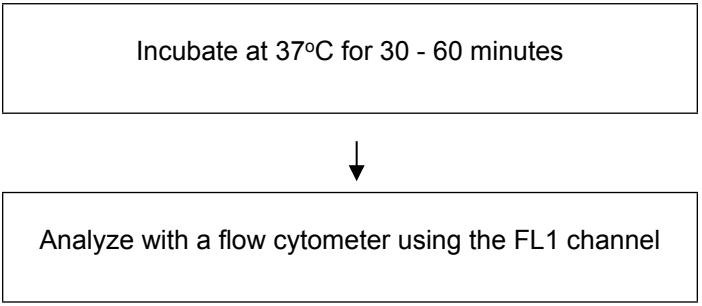
The cell cycle has four sequential phases: G0/G1, S, G2, and M. During a cell's passage through cell cycle, its DNA is duplicated in S (synthesis) phase and distributed equally between two daughter cells in M (mitosis) phase. These two phases are separated by two gap phases: G0/G1 and G2. The two gap phases provide time for the cell to grow and double the mass of their proteins and organelles. They are also used by the cells to monitor internal and external conditions

before proceeding with the next phase of cell cycle. The cell's passage through cell cycle is controlled by a host of different regulatory proteins.

ab112116 is designed to monitor cell cycle progression and proliferation by using our proprietary Nuclear Green CCS1 in live cells. The percentage of cells in a given sample that are in G0/G1, S and G2/M phases, as well as the cells in the sub-G1 phase prior to apoptosis can be determined by flow cytometry. Cells stained with the Nuclear Green CCS1 can be monitored with a flow cytometer at Ex/Em = 490 nm/520 nm (FL1 channel).

## 2. Protocol Summary






*Note: Thaw all the kit components to room temperature before starting the experiment.*

## 3. Kit Contents

| **Components** | **Amount** |
|---|---|
| Component A: 200X Nuclear Green CCS1 | 1 x 250 µL |
| Component B: Assay Buffer | 1 x 50 mL |

## 4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

## 5. Assay Protocol

***Note: This protocol is for each sample.***

**A.** For each sample, prepare cells in 0.5 mL of warm medium or buffer of your choice at a density of 5 x$10^5$ to 1x$10^6$ cells/mL.

*Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.*

**B.** Treat cells with test compounds for a desired period of time to induce apoptosis or other cell cycle functions**.**

***C.*** Add the 200X Nuclear Green CCS1 (component A) to the cell suspension to achieve a final dilution of 1/200 (1X Nuclear Green CCS1). Incubate the cells in a 37 °C, 5% CO2 incubator for 30 to 60 minutes.

*Note 1: For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to incubation with Nuclear Green CCS1.*

*Note 2: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.*

*Note 3: It is not necessary to fix the cells before DNA staining since the Nuclear Green CCS1 is cell- permeable.*

**D.** Wash cells 3x with serum containing growth medium. Centrifuge the cells at 1000 rpm for 4 minutes in between, and finally re-suspend cells in 0.5 mL of Assay Buffer (Component B) or the buffer of your choice.

**E.** Monitor the fluorescence intensity by flow cytometry using the FL1 channel (Ex/Em = 490/525 nm). Gate on the cells of interest, excluding debris.

## 6. Data Analysis

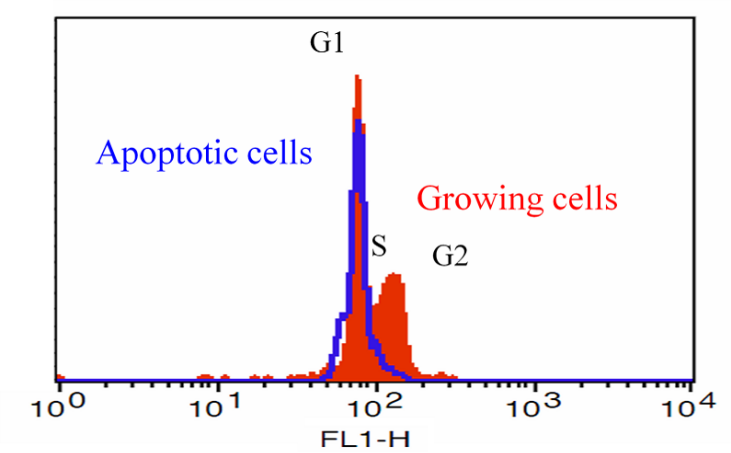



**Figure 1.** DNA profile in growing and camptothecin treated Jurkat cells. Jurkat cells were treated without (red) or with 20 μM camptothecin (blue) in a 37 $^{o}$C, 5% $CO_2$ incubator for about 8 hours, and then dye loaded with Nuclear Green CCS1 for 60 minutes. The fluorescence intensity of Nuclear Green CCS1 was measured with a flow cytometer using the FL1 channel. In growing Jurkat cells, nuclear stained with Nuclear Green CCS1 shows G1, S and G2 phases (red). In camptothecin treated apoptotic cells (B), the fluorescence intensity of Nuclear Green CCS1 was decreased, and both S and G2 phases were diminished.

# 7. Troubleshooting

| Problem | Reason | Solution |
|---|---|---|
| Assay not working | Assay buffer at wrong temperature | Assay buffer must not be chilled - needs to be at RT |
| | Protocol step missed | Re-read and follow the protocol exactly |
| | Plate read at incorrect wavelength | Ensure you are using appropriate reader and filter settings (refer to datasheet) |
| | Unsuitable microtiter plate for assay | Fluorescence: Black plates (clear bottoms);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>If critical, datasheet will indicate whether to use flat- or U-shaped wells |
| Unexpected results | Measured at wrong wavelength | Use appropriate reader and filter settings described in datasheet |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Unsuitable sample type | Use recommended samples types as listed on the datasheet |
| | Readings are outside linear range | Concentrate/ dilute samples or reagents to be in linear range |

| Problem | Reason | Solution |
|---|---|---|
| Samples with inconsistent readings | Unsuitable sample type | Refer to datasheet for details about incompatible samples |
| | Samples prepared in the wrong buffer | Use the assay buffer provided (or refer to datasheet for instructions) |
| | Samples not deproteinized (if indicated on datasheet) | Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)*** |
| | Cell/ tissue samples not sufficiently homogenized | Increase sonication time/ number of strokes with the Dounce homogenizer |
| | Too many freeze-thaw cycles | Aliquot samples to reduce the number of freeze-thaw cycles |
| | Samples contain impeding substances | Troubleshoot and also consider deproteinizing samples |
| | Samples are too old or incorrectly stored | Use freshly made samples and store at recommended temperature until use |
| Lower/ Higher readings in samples and standards | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Out-of-date kit or incorrectly stored reagents | Always check expiry date and store kit components as recommended on the datasheet |
| | Reagents sitting for extended periods on ice | Try to prepare a fresh reaction mix prior to each use |
| | Incorrect incubation time/ temperature | Refer to datasheet for recommended incubation time and/ or temperature |
| | Incorrect amounts used | Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume) |

| | | |
|---|---|---|
| Standard curve is not linear | Not fully thawed kit components | Wait for components to thaw completely and gently mix prior use |
| | Pipetting errors when setting up the standard curve | Try not to pipette too small volumes |
| | Incorrect pipetting when preparing the reaction mix | Always prepare a master mix |
| | Air bubbles in wells | Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates |
| | Concentration of standard stock incorrect | Recheck datasheet for recommended concentrations of standard stocks |
| | Errors in standard curve calculations | Refer to datasheet and re-check the calculations |
| | Use of other reagents than those provided with the kit | Use fresh components from the same kit |

**For technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).**

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