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ab112117

Cell Cycle Assay Kit – Red Fluorometric

Instructions for Use

For monitoring cell cycle progression and proliferation in fixed cells using our proprietary Nuclear Red CCS1

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

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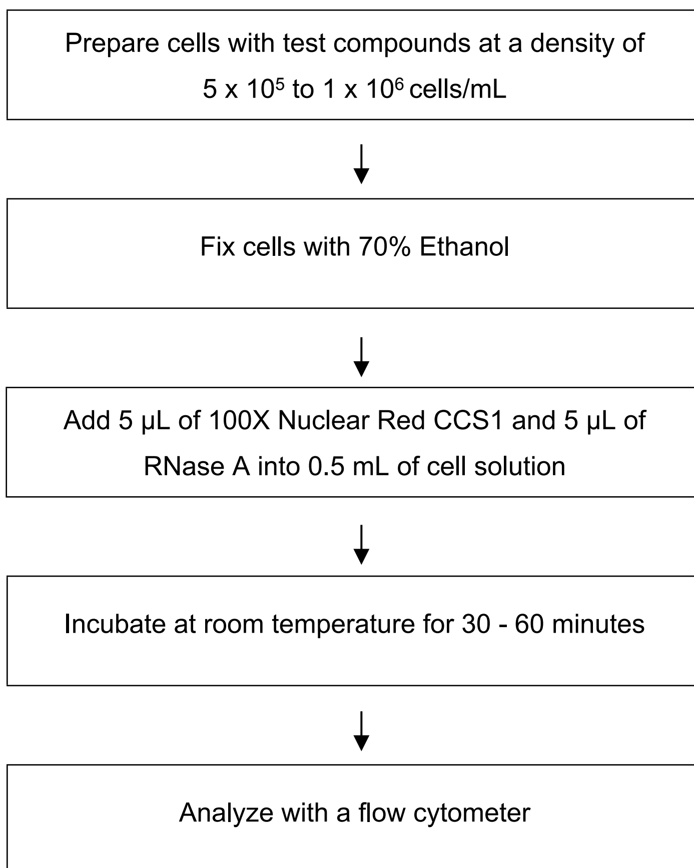
1. Introduction

Abcam Cell Cycle assay kits are a set of tools for monitoring cell viability and proliferation. There are a variety of parameters that can be used. In normal cells, DNA density changes depending on whether the cell is growing, dividing, resting, or performing its ordinary functions. The progression of the cell cycle is controlled by a complex interplay among various cell cycle regulators. These regulators activate transcription factors, which bind to DNA and turn on or off the production of proteins that result in cell division. Any misstep in this regulatory cascade causes abnormal cell proliferation which underlies many pathological conditions, such as tumor formation. Potential applications for live-cell studies are in the determination of cellular DNA content and cell cycle distribution for detecting variations in growth patterns, monitoring apoptosis, and evaluating tumor cell behavior and suppressor gene mechanisms.

ab112117 is designed to monitor cell cycle progression and proliferation by using our proprietary Nuclear Red CCS1 in fixed cells. The dye passes through a permeabilized membrane and intercalates into cellular DNA. The signal intensity of Red Fluorescence is directly proportional to DNA content after RNA is degraded by RNase provided in the kit. 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 monitored with a flow cytometer at Ex/Em = 490/620 nm (FL2 channel).

2. Protocol Summary



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

3. Kit Contents

Components	Amount
Component A: 100X Nuclear Red CCS1	1 x 250 µL
Component B: 100X RNase A	1 x 250 µL
Component C: Assay Buffer	1 x 50 mL

4. Storage and Handling

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

5. Assay Protocol

A. Prepare cells:

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

2. For each sample, prepare cells in 0.5 mL PBS at a density of $5x10^5$ to $1x10^6$ cells/mL.

   Note 1: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

   Note 2: For Adherent Cells - The cells are trypsinized, suspended in 10% FBS medium, centrifuged (1000 rpm, 5 min), and the pellets are re-suspended in PBS.
   For Suspension Cells - The cells are centrifuged (1000 rpm, 5 min), and the pellets suspended in PBS (1 mL).

B. Fix the cells with 70% Ethanol

Pipette 0.5 mL cell suspension (from section A, step 2) into 1.2 mL absolute Ethanol (final concentration approx. 70%). Incubate cells on ice for at least 2 hours (or overnight at -20°C). Cells can be stored at -20 °C for up to 2 years before staining.

Note 1: Ethanol is commonly used for fixation after cell surface antigens were stained with monoclonal antibodies, while methanol is commonly used for fixation after intracellular antigens were stained with monoclonal antibodies.

Note 2: In this procedure whole cells are fixed and analyzed. Because the entire cell mass is still present, the use of RNase is typically included to eliminate any double-stranded RNA. Despite the fact that whole cells are being analyzed, attempts to detect some intracellular antigens in conjunction with DNA may fail because the proteins leak out of the permeabilized cell (e.g. green fluorescent protein). In these cases a brief pre-fixation (10 minutes at 4-6 °C) with 1% paraformaldehyde in PBS before the alcohol fixation will help retain the proteins in the cell.

C. Stain the cells with Nuclear Red CCS1:

1. Pellet the cells at 1000 rpm for 5 minutes (from step B), and wash cells at least once with cold PBS.

2. Suspend the pellet in 0.5 mL of Assay buffer (Component C), and add 5 µL of 100X Nuclear Red CCS1 (Component A) and 5 µL of 100X RNase A (Component B). Incubate the cells at room temperature for 30 to 60 minutes.

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

3. Optional: Centrifuge the cells at 1000 rpm for 5 minutes, and re-suspend cells in 0.5 mL of assay buffer (Component C) or buffer of your choice.

4. Monitor the fluorescence intensity with a flow cytometer using the FL2 channel (Ex/Em = 490/620 nm). Gate on the cells of interest, excluding debris.

6. Data Analysis

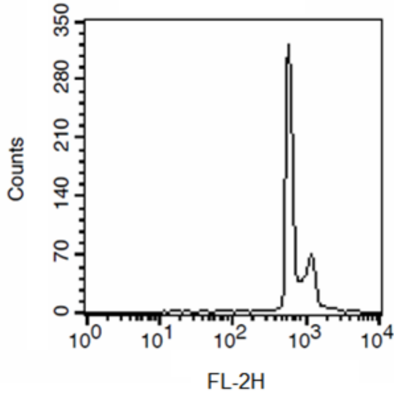


Figure 1. DNA profile in growing Jurkat cells. Jurkat cells were dye-loaded with Nuclear Red CCS1 and RNase A for 30 minutes. The fluorescence intensity of Nuclear Red CCS1 was measured with a flow cytometer using the FL2 channel.

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); Luminescence: White plates; Colorimetry: Clear plates. 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
	Sample readings are outside linear range	Concentrate/ dilute samples 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 further 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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UK, EU and ROW
Email:
technical@abcam.com
Tel: +44 (0)1223 696000
www.abcam.com

US, Canada and Latin America
Email: us.technical@abcam.com
Tel: 888-77-ABCAM (22226)
www.abcam.com

China and Asia Pacific
Email: hk.technical@abcam.com
Tel: 400 921 0189 / +86 21 2070 0500
www.abcam.cn

Japan
Email: technical@abcam.co.jp
Tel: +81-(0)3-6231-0940
www.abcam.co.jp

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