

ab139418 Propidium Iodide Flow Cytometry Kit for Cell Cycle Analysis

To determine cell cycle status in tissue culture cell lines by measuring DNA content using a flow cytometer.

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

[View kit datasheet: www.abcam.com/ab13418](http://www.abcam.com/ab13418)

(use www.abcam.cn/ab13418 for China, or www.abcam.co.jp/ab13418 for Japan)

Storage and Stability

This kit is shipped at +4°C. This kit is stable for at least 6 months from receipt.

Materials Supplied

Item	Quantity	Storage temperature (before prep)
10X Phosphate Buffered Saline (PBS)	100 mL	4°C
20X Propidium iodide (1mg/mL)	2 mL	4°C
200X RNaseA	200 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to perform this assay:

Nanopure water or equivalent.

Ethanol or Methanol

Reagent Preparation

10X Phosphate Buffered Saline (PBS): Prepare an appropriate amount of 1X PBS in nanopure water or equivalent. For example, dilute 5 mL of 10X PBS in 45 mL water. Mix well and store at room temperature.

20X Propidium iodide and 200X RNaseA: Immediately prior to use prepare the Propidium iodide + RNase Staining Solution in PBS. For example, 9.45 mL PBS + 500 µL 20X Propidium iodide + 50 µL 200X RNase.

Δ Note – Propidium iodide is toxic! Handle with care and dispose of according to local regulations.

Sample Preparation

A critical component of any flow cytometry experiment is having a single-cell suspension of the cells being analyzed. Means of achieving single-cell suspensions from adherent cells and suspension cells are detailed below.

Propidium iodide staining is compatible with cells from any species. Cell line selection and any cell treatments are at the discretion of the experimenter.

The volumes given below assume a 6-well plate equivalent of cells (~100,000 – 600,000 cells).

Volumes can be scaled-up proportionately when processing more cells.

Two methods exist for collecting cells; Method I – Collecting Adherent Cells and Method II Collecting Suspension Cells.

1.1 Method I – Collecting Adherent Cells:

1. Culture cells normally.
2. Generate a single cell suspension in a manner similar to routine passaging of the cells: Remove the culture media, rinse the cells with PBS and add trypsin to dissociate cells. Importantly, do not discard the culture media or PBS rinse as they likely contain loosely adherent cells (mitotic or apoptotic) that should be included in analysis. Instead, pool and save the culture media and PBS rinse.
3. Once cells are fully trypsinized (detached from plate and from each other), quench the media with the saved media + PBS wash from the previous step. Gently pipette up and down if necessary, to generate a single cell suspension. Proceed to step 1.3.

1.2 Method II – Collecting Suspension Cells:

1. Culture cells normally.

2. If necessary, gently pipette cells up and down to disperse any cell aggregates.

3. Proceed to step 1.3.

1.3 Sample Preparation Following Cell Collection

1. Pellet the cells at 500 x g for 5 minutes. Aspirate and discard the supernatant.
2. Wash cells with 1X PBS: add 1 mL 1X PBS to the cell pellet, gently resuspend the cell pellet, spin again at 500 x g for 5 minutes.
3. Fix the cells in 66% Ethanol on ice: Gently resuspend the cell pellet in 400 µL ice cold 1X PBS. Slowly add 800 µL ice cold 100% ethanol and mix well. Store at +4°C for at least 2 hours. Cells are stable for at least 4 weeks.

Δ Note: Cell fixation with 66% ethanol or 66% methanol yields similar results (see Figure 3 below) so it is up to the user to choose the alcohol for fixation. We recommend ethanol because it is less toxic for the user and the environment.

Δ Note: At no point should the cells or cell pellets be allowed to dry.

Assay Procedure

- The following propidium iodide staining procedure should be performed immediately before analysis on the flow cytometer. Note that propidium iodide stains DNA in equilibrium with the buffer: cells are analyzed in the propidium iodide staining mixture (the dye is not washed away from the cells).
 - The volumes given below assume ~100,000 – 500,000 cells per tube. Volumes can be adjusted up or down proportionately with cell number.
1. Transfer the previously prepared cells from 4°C to the bench-top and equilibrate to room temperature.
 2. Gently re-suspend cells by inverting the tube or by gentle up and down pipetting. There may be visible thin salt crystals in the tube, but this will not affect the sedimentation of the cells in the next step.
 3. Pellet the cells at 500 x g for 5 minutes. Carefully aspirate the supernatant without disrupting the pellet. It is better to incompletely remove the supernatant than to accidentally aspirate part of the pellet and lose cells.
 4. Wash the cells by gently resuspending in 1 mL 1X PBS. Again, pellet the cells at 500 x g for 5 minutes and carefully remove the supernatant.
 5. Gently resuspend the cell pellet in 200 µL 1X Propidium Iodide + RNase Staining Solution (see 5.2). Ensure that the cells are fully resuspended.
 6. Incubate at 37°C in the dark for 20 – 30 minutes.
 7. Place tubes on ice (still in the dark) and prepare for flow cytometry analysis.
 8. Gently resuspend cells that settled during the incubation by pipetting up and down. If desired, pass cells through an appropriate filter (not provided) to remove cell aggregates.
 9. Run samples on flow cytometer: Set appropriate FSC vs. SSC gates to exclude debris and cell aggregates. Collect Propidium iodide fluorescence in FL2.

Data Analysis

The following are general guidelines. Specific methods of analysis will vary with different flow cytometer analysis programs.

- Establish appropriate FSC vs. SSC gates to exclude debris and cell aggregates. See Figure 1C.
- Collect propidium iodide fluorescence in the appropriate channel (typically FL2) using 488 nm laser illumination.
- Expect to see a 2-fold intensity difference between the 2N and 4N Propidium iodide peaks (plot FL2 with a linear scale on a histogram plot). See Figure 1A.
- Setting up markers on a histogram plot to delineate <2N, 2N, 2N-4N, 4N and >4N intensity regions facilitates quantifying differences in DNA content between samples within an experiment. See Figure 2.
- If applicable, minor voltage adjustments may be required to align 2N peaks between samples.

Assay Performance

Figures 1-4 below show typical results using ab139418 on HeLa and Jurkat cell lines. A useful way to display Propidium iodide data is on a histogram with the cell count on the y-axis and the propidium iodide fluorescence intensity on the x-axis.

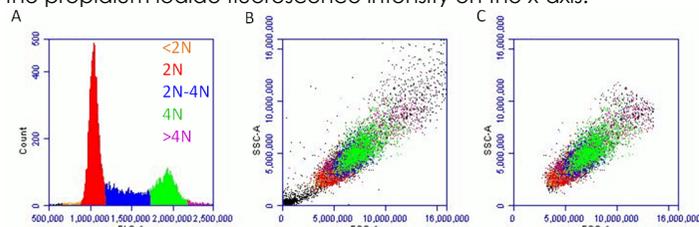


Figure 1. Sample flow cytometry data using ab139418 on untreated HeLa cells. (A) Propidium iodide histogram with DNA content color coded. This is a typical result for untreated healthy cells: most cells are G1 stage (2N), some cells are undergoing DNA synthesis (2N-4N) and the final population is mitotic (4N). Very few cells are apoptotic (<2N) or have excess more than 4N DNA content (>4N). (B) Ungated Forward and Side-scatter plot for HeLa cells in which has been pseudo-colored based on the DNA content shown in (A). Note that cell size is proportional to DNA content. (C) Gated Forward and Side-scatter plot which excludes the small debris (lower left) and cell aggregates (upper right) that are seen in (B). This is the gate used for the Propidium iodide histogram shown in (A).

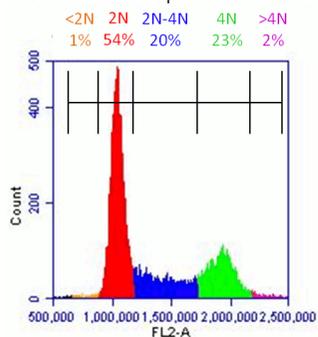


Figure 2. Sample analysis of an experiment using ab139418 on HeLa cells. Use flow cytometer software to establish markers on a histogram plot to quantify the percentage of cells with <2N, 2N, 2N-4N, 4N and >4N DNA content.

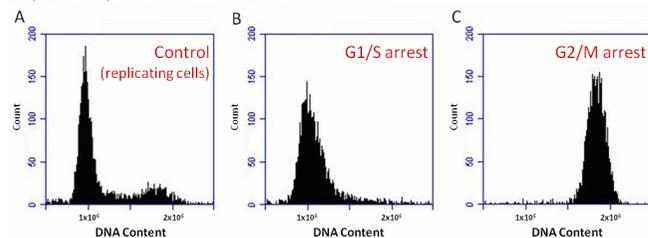


Figure 3. Sample experiment using ab139418 on HeLa cells treated with Thymidine and Nocodazole. (A) Untreated HeLa cells with the expected distribution of cells with 2N, 2N-4N and 4N DNA content. (B) HeLa cells treated with Thymidine have predominantly 2N DNA content. Thymidine inhibits DNA synthesis. (C) HeLa cells treated with Nocodazole are

predominantly have predominantly 4N DNA content. Nocodazole is a microtubule inhibitor that causes mitotic arrest.

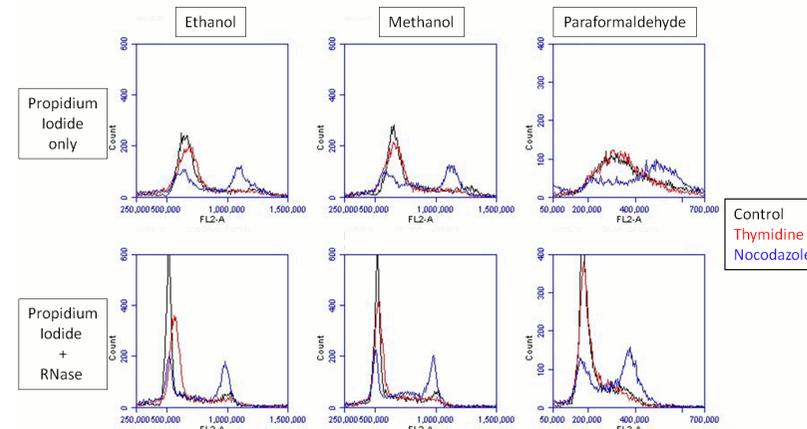


Figure 4. Comparison of fixation and staining conditions using ab139418 on Jurkat cells. Jurkat cells were untreated (Control) or treated with Thymidine or Nocodazole for 24h. Parallel plates of cells were fixed with 66% ethanol, 66% methanol or 4% paraformaldehyde; each preparation was then stained with propidium iodide with or without the addition of RNase. For each fixation condition, treatment with RNase yields tighter propidium iodide peaks. Moreover, ethanol and methanol fixation yield superior propidium iodide data relative to paraformaldehyde fixation.

FAQs

How many cells do I need to analyze?

A general guideline is to analyze 10,000 cells per sample. Generally, there is some cell loss during the fixation and staining procedure, so it is recommended to harvest at least 10 times that many cells. The assay will perform well in the range of 500,000 – 2,500,000 cells/mL in the Propidium iodide + RNase staining solution. Adjust the volume of the Propidium iodide + RNase staining solution appropriately to achieve this concentration range.

What is the relationship between DNA content and cell cycle?

Cells with two copies of each chromosome (2N) are not actively in the process of cell replication and are in one of two states: G1 (not currently replicating but retain the ability to replicate) or G0 (senescent). Cells that are in the process of replicating and are actively copying their DNA have an amount of DNA that is intermediate between 2N and 4N (2N – 4N). After DNA synthesis is completed the DNA content is doubled (4N) and cells are either in a resting state (G2) or in mitosis (M). For more information find the cell cycle resource at www.abcam.com.

Can I combine Propidium iodide staining with an antibody stain?

In principle the answer is yes, but there are not definitive guidelines as all depends on the individual antibody that one wants to use. Optimization steps include testing the antibody staining with different fixation methods (e.g. Figure 4). Generally, antibody staining should be performed first and followed by Propidium iodide staining (the Propidium iodide staining should be done at room temperature rather than at 37°C). Use a directly fluorescently conjugated primary antibody when possible. Finally, care must be taken in choosing the most appropriate fluorescent label for the antibody as the Propidium iodide emission spectrum is broad. FITC or similar is recommended.

Technical Contacts

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)
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