

Cell Cycle Assay Solution Deep Red

Cell Cycle Assay Solution Blue

Technical Manual

Technical Manual (Japanese version) is available at <http://www.dojindo.co.jp/manual/C548, C549.pdf>

General Information

Cell cycle is associated with the most important phenomena of cell growth. Various phases of the cell cycle can be analyzed by staining DNA and using a flow cytometer. Propidium Iodide (PI) is a widely used dye for cell cycle analysis using a flow cytometer. However, PI has a number of limitations because of the following reasons.

- 1) Cell fixation is required (non-cell membrane permeability)
- 2) RNase treatment is needed (PI is non-selective toward DNA)
- 3) Necessity of compensation (PI is detected by the PE channel and when using antibody conjugated dye detected FITC, compensation is necessary).

The Cell Cycle Assay Solution overcomes these limitations. This solution offers a much simpler procedure when compared with that using PI. Two different color options (Deep Red and Blue) are provided as ready-to-use solutions. Furthermore, either solution enables the determination of the ratio of the G0/G1 phase, S phase, and G2/M phase using a flow cytometer. The color of either solution can be combined with your experiments.

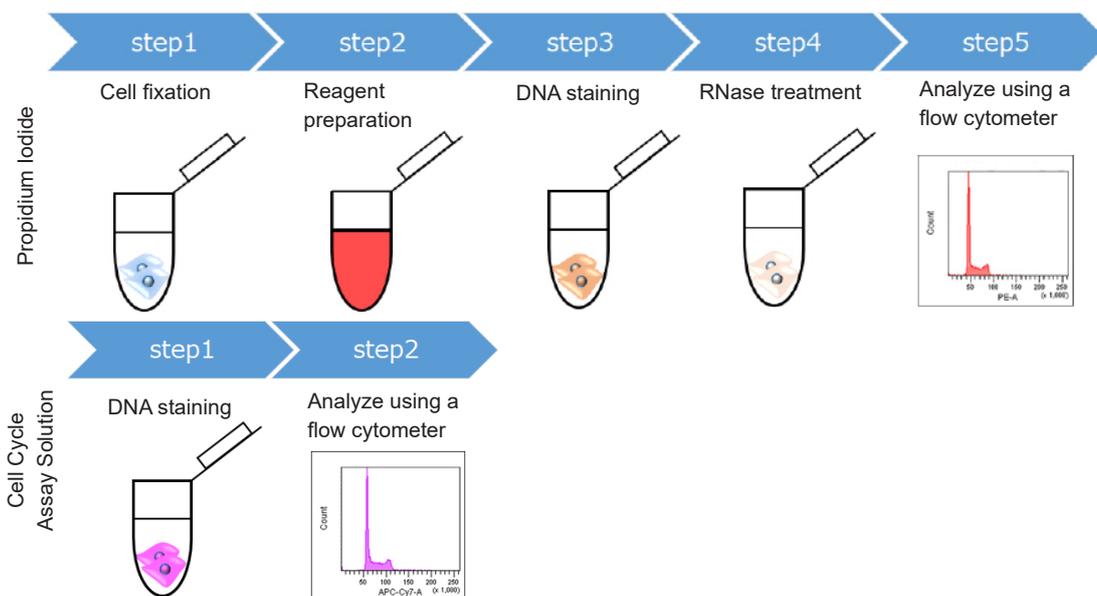


Figure 1 The step-by-step procedures for measuring the cell cycle with PI and the Cell Cycle Assay Solution.

Contents

Cell Cycle Assay Solution Deep Red
Cell Cycle Assay Solution Blue

Code	50 tests
C548	250 μ l \times 1
C549	250 μ l \times 1

*C548 and C549 are provided as aqueous solutions.

Storage Condition

C548 Store at -20°C and protected from light.
C549 Store at -20°C .

Required Equipment and Materials

- Flow cytometer
- Cell strainer for flow cytometer
- Incubator (37°C)
- Micropipettes (100–1000 μ l, 1–20 μ l)
- Conical tubes
- Microcentrifuge tubes
- Phosphate buffered saline (PBS)

Preparation

Allow the Cell Cycle Assay Solution to equilibrate at room temperature before use.

*Centrifuge the tube briefly before opening the cap because the content may be on the tube wall or on the ceiling of the cap.

Precaution

Optimize the gating (FSC, SSC) after the staining because the scattergram on FSC and SSC are changed by the staining process with the Cell Cycle Assay Solution.

*Refer Figure 5.

Remaining trypsin may result obtaining improper histogram in Flow cytometric analysis.
Carefully remove trypsin by washing before staining.

*Refer Figure 6.

Too much number of cells may cause precipitation in the sample.
Optimization of cell number is required.

*Refer Figure 7.

For non-fixed cells

1. Prepare a cell suspension ($1-5 \times 10^5$ cells/ml) in a 1.5 ml microcentrifuge tube.
2. Centrifuge at $300 \times g$ for 5 minutes and discard the supernatant.
3. Add 500 μ l of PBS to each microcentrifuge tube, suspend by pipetting, centrifuge at $300 \times g$ for 5 minutes and discard the supernatant.
4. Add 500 μ l of PBS to each microcentrifuge tube and add 5 μ l of Cell Cycle Assay Solution.
5. Incubate at 37°C for 15 minutes and protect from light.
6. Pass through a cell strainer and analyze samples using a flow cytometer.

*Recommended filter settings are shown in Table 1.

For fixed cells

1. Prepare a cell suspension ($1-5 \times 10^5$ cells/ml) in a 1.5 ml microcentrifuge tube.
2. Centrifuge at $300 \times g$ for 5 minutes and discard the supernatant.
3. Fix the cells by using one of the following protocols or your original fixation protocol.
4. Add 5 μ l of C548 or C549 and incubate at 37°C for 15 minutes, protect from light.
5. Pass through a cell strainer and analyze samples using a flow cytometer.

*Recommended filter settings are shown in Table 1.

Using a 4% paraformaldehyde (PFA) /PBS solution.

1. Add 1 ml of a 4% PFA/PBS solution to a microcentrifuge tube and incubate at room temperature for 20 minutes.
2. Centrifuge at $300 \times g$ for 5 minutes and discard the supernatant.
3. Add 500 μ l of PBS to the microcentrifuge tube, suspend by pipetting, centrifuge at $300 \times g$ for 5 minutes. Discard the supernatant and add 500 μ l of PBS to each microcentrifuge tube.

Using cold 70% ethanol.

1. Add 1 ml of a 70% ethanol to a microcentrifuge tube and incubate at -20°C for 120 minutes.
2. Centrifuge at $300 \times g$ for 5 minutes and discard the supernatant.
3. Add 500 μ l of PBS to the microcentrifuge tube, suspend by pipetting, centrifuge at $300 \times g$ for 5 minutes. Discard the supernatant and add 500 μ l of PBS to each microcentrifuge tube.

Code	Excitation (nm)	Emission (nm)
C548	633–647 nm	695LP or 780/60 BP *ex.) APC-Cy7 (BD)
C549	405–407 nm	450/50 BP *ex.) Pacific Blue (BD)

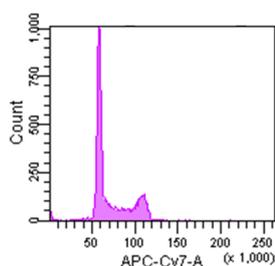
* LP: Long pass filter
* BP: Band pass filter

Table 1 Recommended filter settings of the Cell Cycle Assay Solution.

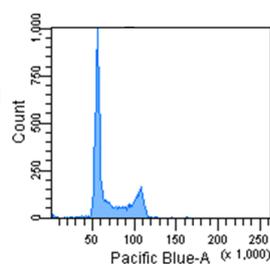
Usage examples

Cell Cycle Analysis using HeLa cells

1. HeLa cells in MEM (containing 10% fetal bovine serum, 1% penicillin-streptomycin) were seeded on a 6-well plate ($1-5 \times 10^5$ cells/ml) and cultured overnight in a 37°C incubator equilibrated with 95% air and 5% CO₂.
2. After trypsinization, 1 ml of the cell suspension was transferred to a microcentrifuge tube.
3. The cells suspension was centrifuged at $300 \times g$ for 5 minutes.
4. The supernatant was discarded and 500 μ l PBS was added to the microcentrifuge tube and suspended by pipetting.
5. The cells suspension was centrifuged at $300 \times g$ for 5 minutes and the supernatant was discarded.
6. The cells were re-suspended in PBS (500 μ l) and 5 μ l of C548 or C549 was added.
7. Each tube was incubated at 37°C for 15 minutes, protected from light.
8. The stained cells were passed through a cell strainer and analyze samples using a flow cytometer.



Cell Cycle Assay Solution Deep Red
Excitation: 640 nm
Emission: 780/60 nm



Cell Cycle Assay Solution Blue
Excitation: 405 nm
Emission: 450/50 nm

Figure 2 Cell cycle analysis using HeLa cells stained with the Cell Cycle Assay Solution.

Cell Cycle Analysis using Jurkat cells

1. Jurkat cells in MEM (containing 10% fetal bovine serum, 1% penicillin-streptomycin) were seeded on a 6-well plate ($1-5 \times 10^5$ cells/ml) and cultured overnight in a 37°C incubator equilibrated with 95% air and 5% CO₂.
2. After trypsinization, 1 ml of the cell suspension was transferred to a microcentrifuge tube.
3. The cells suspension was centrifuged at $300 \times g$ for 5 minutes.
4. The supernatant was discarded and 500 μ l PBS was added to the microcentrifuge tube and suspended by pipetting.

5. The cells suspension was centrifuged at $300\times g$ for 5 minutes and the supernatant was discarded.
6. The cells were re-suspended in PBS (500 μ l) and 5 μ l of C548 or C549 was added.
7. Each tube was incubated at 37°C for 15 minutes, protected from light.
8. The stained cells were passed through a cell strainer and analyze samples using a flow cytometer.

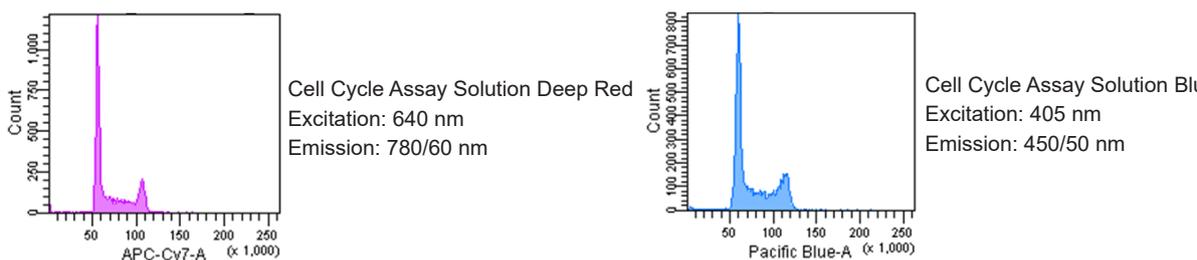


Figure 3 Cell cycle analysis using Jurkat cells stained with the Cell Cycle Assay Solution.

Troubleshooting

Optimize the gating (FSC, SSC) after staining because the FSC and SSC signals are changed by the staining.

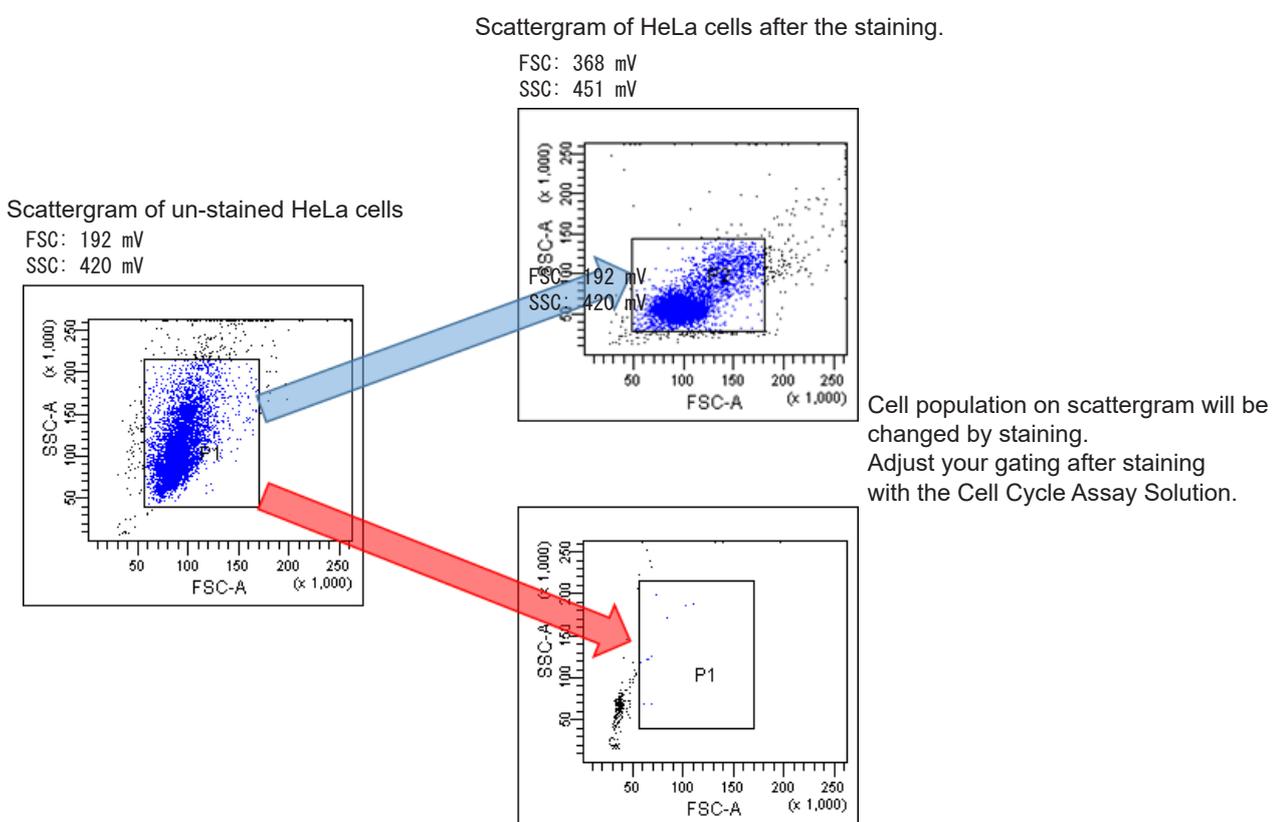


Figure 5 Scattergram of HeLa cells before or after staining.

Negative effect of trypsin

Carefully remove trypsin by washing. Remaining trypsin may result obtaining improper histogram as follows.

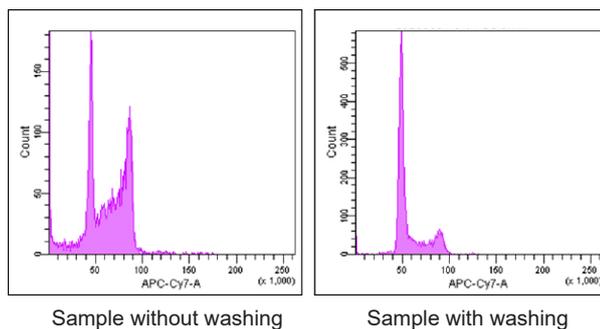
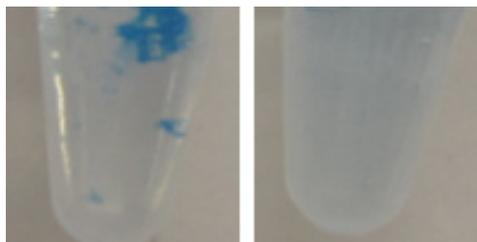


Figure 6 Histogram of HeLa cells in the presence/absence of trypsin.

Precipitates in the sample

When precipitates are observed, reduce the number of cell in the sample.

Precipitates in the sample No precipitates in the sample



8.4×10^5 cells/ml

4.2×10^5 cells/ml

cell line	HeLa
cell number	$4.2\text{--}8.4 \times 10^5$ cells/ml
amount of reagent	5 μ l
incubation	37°C , 15 minutes

Figure.7 Precipitates in the sample of HeLa cells.

*For research use only. Not for use in diagnostic procedures.
If you need more information, please contact Dojindo technical service.*

Dojindo Laboratories

2025-5 Tabaru, Mashiki-machi, Kamimashiki-gun, Kumamoto
861-2202, Japan Phone: +81-96-286-1515 Fax: +81-96-286-1525
E-mail: info@dojindo.co.jp Web: www.dojindo.co.jp

Dojindo Molecular Technologies, Inc.

Tel: +1-301-987-2667 Web: <http://www.dojindo.com/>

Dojindo EU GmbH

Tel: +49-89-3540-4805 Web: <http://www.dojindo.eu.com/>

Dojindo China Co., Ltd

Tel: +86-21-6427-2302 Web: <http://www.dojindo.cn/>

Tel: +86-10-8225-1765

C548 : Cell Cycle Assay Solution Deep Red
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