molecular probes®
by life technologies™

Tali® Cell Cycle Kit

Catalog no. A10798

Table 1 Contents and storage

Material	Amount	Storage	Stability
Tali® Cell Cycle Solution	10 mL	• Room temperature • DO NOT FREEZE • Protect from light	When stored as directed, kit components are stable for at least 6 months.
Number of reactions: Sufficient material is supplied for 50 assays, based on the protocol below.			
Approximate fluorescence excitation and emission maxima: 535/617 in nm, bound to DNA.			

Introduction

Quantification of cellular DNA content is a commonly used method for monitoring cell cycle progression. As cells progress through the cell cycle, the amount of DNA ultimately doubles. This doubling can be tracked and used to determine the cell cycle phase (G_1, S, and/or G_2/M). Cells in the G_1 phase have one set of paired chromosomes. During the S phase cellular DNA begins to double, so that the amount of DNA is between one and two times the amount in G_1. Cells in G_2/M phase have double the amount of DNA compared to cells in G_1 and two sets of paired chromosomes. Thus, the amount of DNA in cells directly reflect which cell cycle phase the cells are in.

The Tali® Cell Cycle Kit provides an easy-to-use, optimized, all-in-one solution containing propidium iodide, RNase A, and Triton® X-100 to label cells for cell cycle analysis using the Tali® Image-Based Cytometer.

Before You Begin

Materials Required but not Provided

- Cells of interest in single cell suspension (appropriate sample concentrations range from 1×10^5–5×10^6 cells/mL in the assay)
- Dulbecco's Phosphate Buffered Saline (DPBS)
- Ice cold 70% ethanol in distilled water for cell fixation
- Tali® Cellular Analysis Slides (Cat. nos. T10794, T10795)

Caution

Tali® Cell Cycle Solution contains propidium iodide, which is a potential mutagen; use appropriate precautions when handling this reagent.

Dispose of the reagents in compliance with all pertaining local regulations. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Always wear suitable laboratory protective clothing and gloves when handling these reagents.

Storage and Handling

Upon receipt, store the Tali® Cell Cycle Solution at room temperature (2–25°C), protected from light. Do not freeze. When stored properly, the the Tali® Cell Cycle Solution is stable for at least 6 months. This kit contains sufficient material to assay 50 samples using the method outlined below.

Experimental Protocols

Follow the instructions below to perform the Tali® Cell Cycle Assay. For detailed instructions on using the Tali® Image-Based Cytometer, refer to the user guide supplied in the Tali® Image-Based Cytometer USB Drive. The user guide is also available for downloading at www.lifetechnologies.com/tali.

The Tali® Cell Cycle Assay has a final volume of 200 µL and appropriate cell concentrations for the assay range from 1×10^5–5×10^6 cells/mL (i.e., 2×10^4–1×10^6 cells in 200 µL of 70% ice-cold ethanol). If you have greater than 1×10^6 cells, fix the cells to a final concentration of 1×10^5–5×10^6 cells/mL, and then use a 200-µL aliquot of the cell suspension for the Staining Protocol.

Prepare Cells for Labeling

1.1 Wash the cells.

a. Centrifuge the cells at 500 × *g* for 5 minutes.

b. Discard the medium and gently resuspend the cells in DPBS.

c. Centrifuge the cells 500 × *g* for 5 minutes and transfer the tubes to ice.

1.2 Fix the cells with ice-cold 70% ethanol in distilled water.

a. Remove the DPBS.

b. Slowly resuspend the cells in 70% ice-cold ethanol.

Note: It is very important that the cells are fixed into a single cell suspension. Cells can tend to clump during fixation. Very slow, drop-wise addition of the initial volume of 70% ethanol while gently vortexing helps prevent cells from clumping.

c. Place cells at –20°C overnight. These cells can be kept for weeks at –20°C before staining and analysis.

Stain Cells

2.1 Wash the cells.

a. Centrifuge the cells at 1000 × *g* for 5 minutes at 4°C.

b. Remove the ethanol and resuspend cells in 1 mL of DPBS.

c. Centrifuge cells at 500 × *g* for 10 minutes at 4°C.

2.2 Stain the cells with the Tali® Cell Cycle Solution.

a. Remove DPBS and resuspend the cells in 200 µL of Tali® Cell Cycle Solution to a final cellular concentration of 1×10^5–5×10^6 cells/mL (i.e., 2×10^4–1×10^6 cells in 200 µL of Tali® Cell Cycle Solution).

b. Incubate the cells at room temperature for 30 minutes, in the dark.

c. Vortex the cells briefly to gently resuspend them before cell cycle analysis using the Tali® Image-Based Cytometer.

Run Samples

For detailed instructions on using the Tali® Image-Based Cytometer, refer to the user guide supplied in the Tali® Image-Based Cytometer USB Drive. The user guide is also available for downloading at www.lifetechnologies.com/tali.

3.1 Load 25 µL of the stained cells into a Tali® Cellular Analysis Slide by pipetting the sample at an angle of approximately 80° into the half moon-shaped sample loading area. The sample is loaded into the chamber through capillary action. Take care to avoid forming bubbles in the sample or to cause back splatter.

3.2 Insert the slide into the slide port of the Tali® Image-Based Cytometer until it stops. Do not forcefully push the slide any further.

3.3 Touch **Cell Cycle** on the Home screen of the Tali® Image-Based Cytometer to select the Tali® Cell Cycle Assay. Name the sample series, if desired.

3.4 Touch **Press to insert new sample**; the slide will automatically be pulled into the instrument.

3.5 When prompted, focus your cells using the image adjustment (focus) knob on the right side of the instrument.

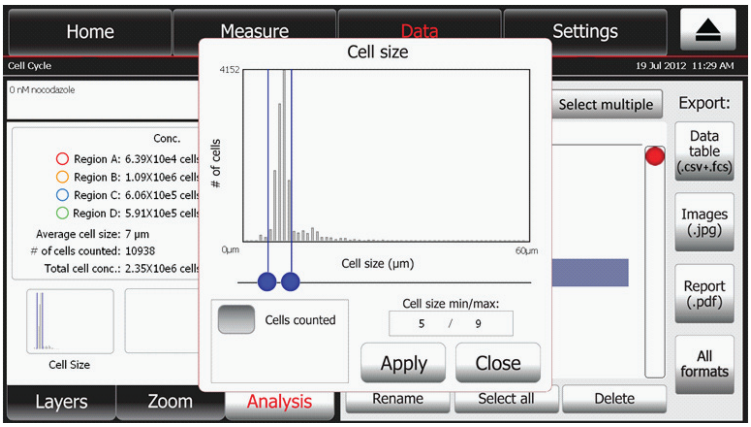
3.6 Specify the number of fields of view to capture using the **# of images to capture** drop-down menu, and then touch **Press to run sample**. The Tali® Image-Based Cytometer will automatically capture and analyze the images of your sample, and present the results of the analysis in the analysis window.

Note: We recommend that you collect 20 fields per sample. Collecting a large number of fields per sample results in more robust data.

Analyze Data

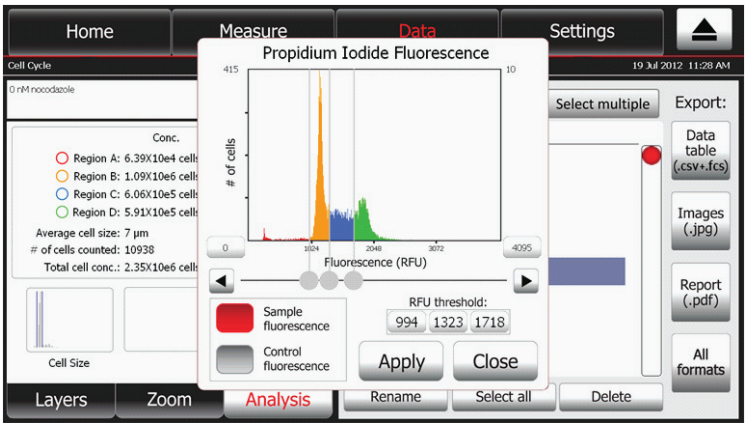
Data analysis can be performed under the Measure Tab during data acquisition or under the Data Tab after data acquisition for all the samples have been completed. Instructions here show screenshots from the Data Tab, but the procedure is the same as the one used during data acquisition.

4.1 Using an untreated sample (one with a normal cell cycle histogram), gate on the cell size. In the example below, small cells (which are debris) and large cells (which are aggregates) are gated out of the analysis.



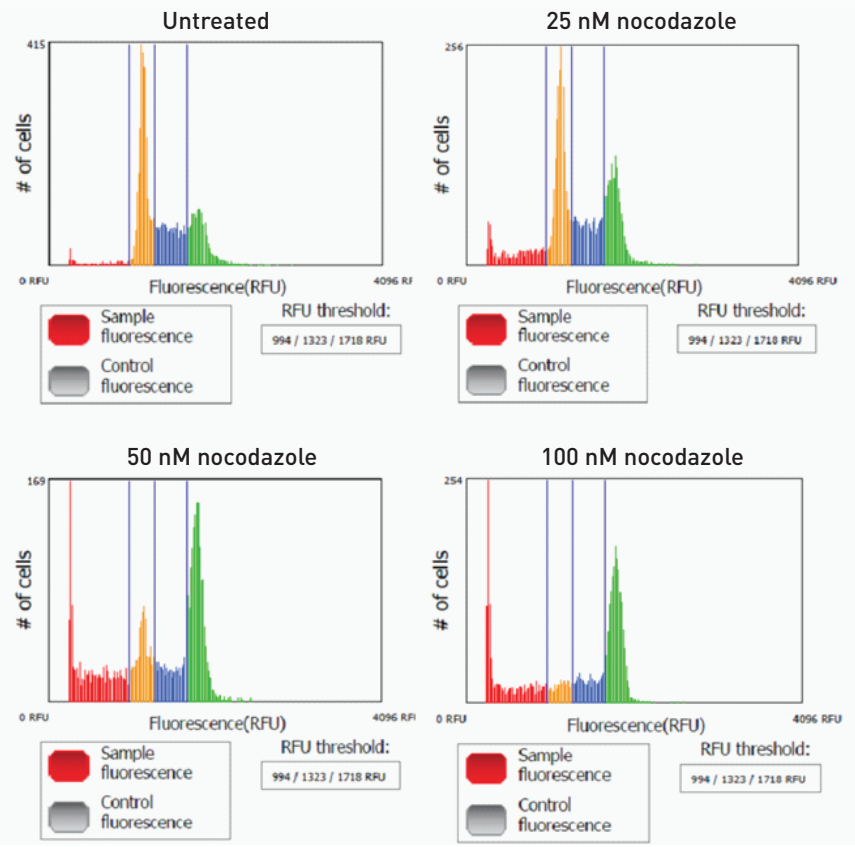
4.2 Next, set the threshold gates for each cell cycle phase. This can be done three ways:

- Tap to select one of the three threshold bars (grey vertical bar on histogram). The selected threshold bar will turn from grey to blue. Tap on arrows at the bottom of the histogram to move threshold bar.
- Tap to select one of the three threshold bars. The selected threshold bar will turn from grey to blue. Drag threshold bar to desired location.
- Tap on the numbers under 'RFU threshold,' a pop-up keyboard will appear, and values can be entered manually.



4.3 Export the data using the Export function available in the Data Tab. You may choose to export raw data (in .csv and .fcs formats), individual images, or the PDF report to a USB flash drive. Analysis using cell cycle modeling software (such as ModFit LT™ or MultiCycle®) to obtain accurate estimates of the percent of cells in each phase of the cell cycle is recommended, especially for arrested or non-normal cell cycle histograms.

Figure 1 Example of cell cycle data analysis. In the example below, Jurkat cells were treated with increasing concentrations of nocodazole to induce G_2 arrest. The RFU threshold was set using the untreated sample and held constant across drug treated samples. Note that, in some cases, the RFU thresholds may need to be slightly adjusted for each sample because peaks may shift with drug treatment.



References

1. Curr Protoc Cell Biol. Ch 8:Unit 8.4 (2001); **2.** Methods Mol Biol. 281, 301 (2004); **3.** Curr Issues Mol Biol. 3, 67 (2001).

Product List

Current prices may be obtained from www.lifetechnologies.com or from our Customer Service Department.

Catalog no.	Product Name	Unit Size
A10798	Tali® Cell Cycle Kit *for use with the Tali® Image-Based Cytometer* *50 assays*	1 kit
Related Products		
T10794	Tali® Cellular Analysis Slides, 50 slides	1 each
T10795	Tali® Cellular Analysis Slides, 500 slides	1 each
T10796	Tali® Image-Based Cytometer	1 unit

Purchaser Notification

Corporate Headquarters
5791 Van Allen Way
Carlsbad, CA 92008
USA
Phone: +1 760 603 7200
Fax: +1 760 602 6500
Email: techsupport@lifetech.com

European Headquarters
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF
UK
Phone: +44 141 814 6100
Toll-Free Phone: 0800 269 210
Toll-Free Tech: 0800 838 380
Fax: +44 141 814 6260
Tech Fax: +44 141 814 6117
Email: euroinfo@invitrogen.com
Email Tech: eurotech@invitrogen.com

Japanese Headquarters
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Japan
Phone: +81 3 5730 6509
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Email: jpinfo@invitrogen.com

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