Phase-Flow™

BrdU Cell Proliferation Kit for Flow Cytometry Manual

Cat. No. 370704
370706

This Phase-Flow™ BrdU Kit is designed for identifying and studying proliferating cells using flow cytometric analysis.

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

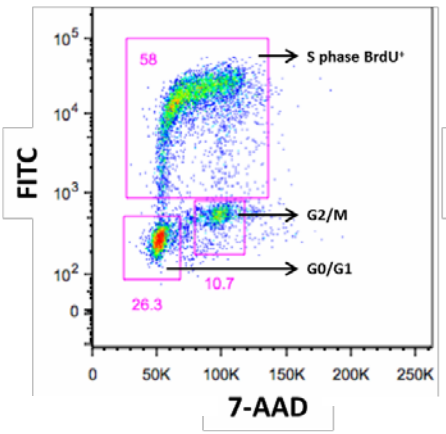
Introduction

Description

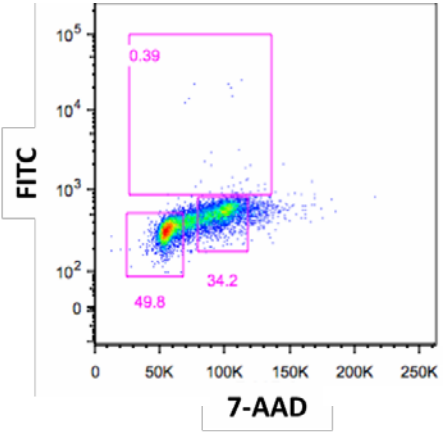
BioLegend's BrdU Kit is designed for studying cell proliferation by staining BrdU (5-bromo-2'-deoxyuridine) that was incorporated into cells with newly synthesized genomic DNA during the S-phase of mitosis and performing flow cytometric analysis. 7-AAD staining is performed in conjunction with immunofluorescent BrdU to help with identification and characterization of cells. Pulse labeling BrdU-containing cells at different time points allows for analysis of cell cycle kinetics.

Representative Data

HEL cells pulsed with or without BrdU for 1 hour and then stained with Phase-Flow™ BrdU Cell Proliferation kit.



HEL cells pulsed with BrdU then stained with kit components for anti-BrdU FITC and 7-AAD



HEL cells without BrdU pulse then stained with kit components for anti-BrdU FITC and 7-AAD.

Kits Content and Storage

Reagent	Concentration/ Amount	Amount to Use	Storage Conditions
BrdU pulsing solution	10 mg/ml (2 vials, 500 µl per vial)	For *in vivo* use, 1 mg/mouse injected ip. For *in vitro* use, 0.5 µl per ml of cell suspension	Freeze at -70°C upon arrival.
Anti-BrdU antibody	100 tests (1 vial)	5 µl per sample	4°C
Buffer A	20 ml	100 µl per sample	4°C
Buffer B	50 ml (10X)	Dilute 1X with DI H_2O. Use 1 ml per sample	4°C
Buffer C	10 ml	100 µl per sample	4°C
DNAse (lyophilized)	400 µg/vial (5 vials)	50 µl per sample. Use 1 ml of PBS (Ca/Mg^{2+}) to reconstitute 1 vial of DNAse. 1 reconstituted vial is enough for 20 samples)	4°C
PBS (Ca/Mg^{2+})	5 ml	1 ml per vial of DNAse	4°C
7-AAD	2.5 ml	20 µl per sample	4°C
DAPI	100 µg (1 vial, lyophilized)	1 µg per sample Dilute stock to 1 mg/ml with DI H_2O, add 1 µl per sample	4°C

Notes: Once thawed, the BrdU pulsing solution is stable between 2°C and 8°C for 4 weeks.
Mix Buffer A by gently inverting the bottle 2 times.

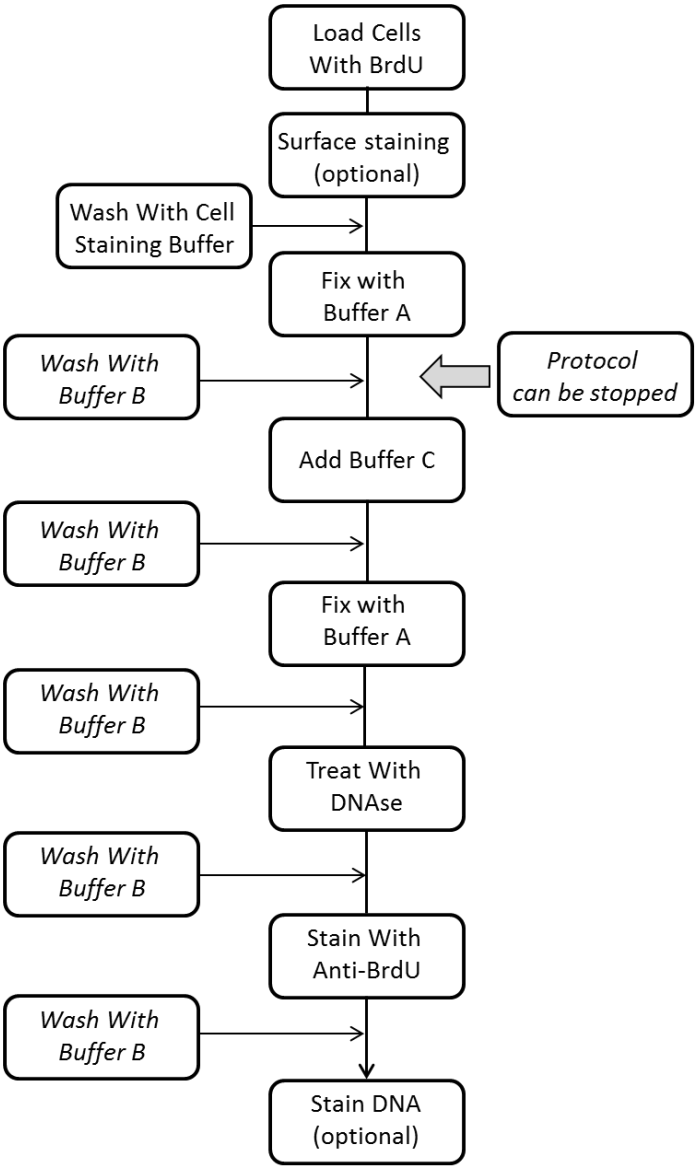
All reagents are guaranteed for 12months.

Reagents not provided but required:

1. Cell Staining Buffer (BioLegend Cat# 420201), or equivalent.
2. DI H_2O

Instructions

Procedure Overview



Assay Procedure

Load and Stain Cells with BrdU

1. Load cells with BrdU. Add BrdU solution at 0.5µl/ml of cell suspension or inject a mouse ip (intraperitoneally) with 1 mg of BrdU. Pulse time should be determined by the researcher. Standard pulse time for cell lines is 1-2 hrs. Prolonged BrdU incubation (e.g. >48 hours) can cause it to cycle out of actively proliferating cells.
2. Harvest cells and wash with Cell Staining Buffer (BioLegend Cat# 420201) or equivalent. Centrifuge for 5 minutes at 200-300xg and discard supernatant.
3. Adjust cell concentration to 1 x 10^7 cells/ml. Aliquot 0.5-1.0 x 10^6 cells/tube (12x75 mm tube recommended).
4. Optional: stain cells with surface antibodies. (Some antibodies for cell surface markers do not recognize fixed epitopes).
5. Wash with Cell Staining Buffer by adding 1 ml/tube and centrifuge for 5 minutes at 200-300xg and discard supernatant. Always leave a residual volume of 50-100µl in the tube. Resuspend cell pellet.

Fix Cells

6. Fix cells by adding 100µl of Buffer A to each tube, mix and incubate on ice or at 4°C for 15-20 minutes.

   Note: Cells can be bulk fixed for staining at a later time. To bulk fix the cells, add 1 ml of Buffer A for each 1 x 10^7 cells and incubate at 4°C for 15-20 minutes. Wash cells with 10 ml of Cell Staining Buffer and freeze them at -80°C in freezing medium (e.g. 90% FCS and 10% DMSO). Cells may be kept at -80°C for a prolonged period of time. To thaw the cells, place them in a 37°C water bath, wash with 10 ml of 1X Buffer B and centrifuge for 5 minutes at 200-300xg, discard supernatant. Continue with the procedure at step 10.

Cell Permeabilization

7. Wash cells using 1 ml of 1X Buffer B, centrifuge for 5 minutes at 200-300xg and discard supernatant. Leave a residual volume of

50-100µl in the tube.

8. Add 100µl per tube of Buffer C. Incubate at room temperature for 10 minutes.
9. Repeat step 7.

Repeat Fixation of Cells

10. Add 100µl per tube of Buffer A and incubate at room temperature for 5 minutes.
11. Repeat step 7.

Treat with DNAse

12. Reconstitute DNAse with 1 ml of PBS (Ca/Mg^{2+}) per vial. DO NOT VORTEX. Allow DNAse to disolve for 2-3 minutes. Add 50µl of DNAse solution to each tube. Incubate for 1 hour in a 37°C incubator or water bath. Leftover DNAse may be stored at -80°C.
13. Repeat step 7.

Stain Cells with anti-BrdU Antibody

14. Add 5µl of anti-BrdU antibody (and other fluorescent antibodies if desired) to the tubes. Incubate for 15-20 minutes at room temperature in the dark.
15. Repeat step 7.
16. Optional: Re-suspend cells in Cell Staining Buffer containing a DNA dye (DAPI 1µg/sample) or 7-AAD (1µg/sample). Incubate for 5-10 minutes prior to acquiring on a flow cytometer.

    Note: If you are staining with Brilliant Violet™ 421 conjugated anti-BrdU antibody, do not use DAPI for DNA staining. If you are staining with PerCP/Cy5.5, PE/CY7 or PE/Cy5 conjugates, do not use 7-AAD for DNA staining.

Product sold by **BioLegend Inc.**
9727 Pacific Heights Blvd. San
Diego, CA 92121
Tel: 1.858.768.5800
Tel: US & Canada Toll-Free: 1.877.Bio-Legend (1.877.246.5343) Fax:
1.877.455.9587
Email:info@biolegend.com
biolegend.com

For a complete list of world-wide BioLegend offices and distributors, please visit our website at: biolegend.com

370704/370706 V1