Technical Data Sheet

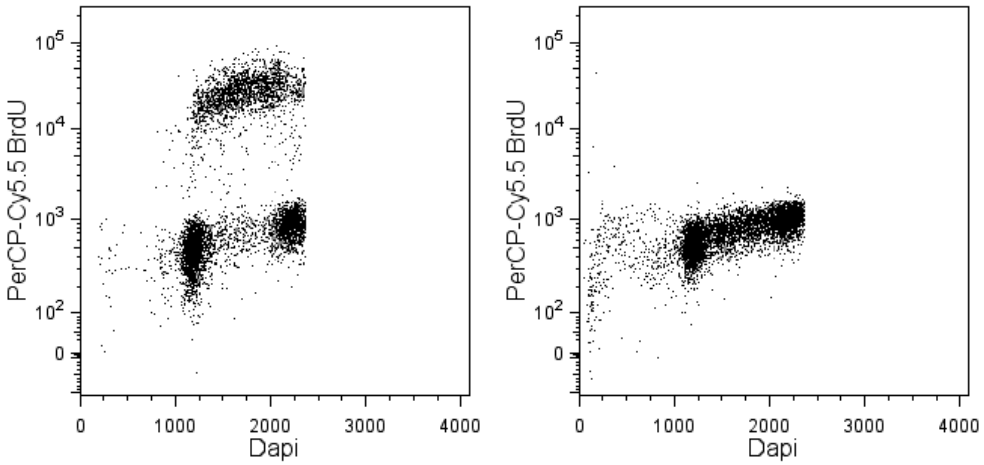
Permeabilization Buffer Plus

Product Information

Material Number:	**561651**
Size:	10 mL
Concentration:	1X
Storage Buffer:	Aqueous buffered solution containing proprietary ingredients.

Description

BD Cytoperm™ Permeabilization Buffer Plus is specially formulated for the immunofluorescent staining of incorporated BrdU for flow cytometric analysis. It is used as a staining enhancer and secondary permeabilization reagent. BD Cytoperm™ Permeabilization Buffer Plus should be used with fixed cell samples only. Use of this buffer on unfixed cells will cause cell damage.



Flow cytometric analysis of DNA synthesis by TK-1 cells. *TK-1 cells were either pulsed with 50 µM BrdU for 1 hour (left panel) or were not pulsed (right panel). Staining was performed using BD Cytoperm™ Permeabilization Buffer Plus in the procedure from the BD Pharmingen™ FITC and APC BrdU Flow Kits. The permeabilized cells were stained with the PerCP-Cy™5.5 Mouse Anti-BrdU monoclonal antibody (Cat. No. 560809) followed by the DNA-specific dye, DAPI dihydrochloride at 1 µg/mL (Sigma, Cat. No. D9542). Two-color flow cytometric dot plots showing the correlated expression patterns of DAPI vs BrdU were derived from gated events with the forward and side light-scatter characteristics of viable lymphocytes. Flow cytometry was performed with doublet discrimination using a BD™ LSRII system.*

Preparation and Storage

Store undiluted at 4°C.

Irritating to eyes and skin. Do not breathe vapor. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

BD Cytoperm™ Permeabilization Buffer Plus is specially formulated for the immunofluorescent staining of incorporated BrdU for flow cytometric analysis and may be found in the BD Pharmingen™ FITC BrdU Flow Kit (Cat. No. 559619 / 557891) or the BD Pharmingen™ APC BrdU Flow Kit (Cat. No. 552598 / 557892). Investigators may find the following abbreviated protocol to be helpful.

1. **Immunofluorescent staining of cell surface antigens.**
   a. Add BrdU-pulsed cells (10^6 cells in 50 µL of staining buffer) to flow cytometry tubes.
   b. Add fluorescent antibodies specific for cell-surface markers in 50 µL of staining buffer (eg, BD Pharmingen™ Stain Buffer (FBS) Cat. No. 554656) per tube and mix well.
   c. Incubate cells with antibodies for 15 minutes on ice.
   d. Wash cells 1x by adding 1 mL of staining buffer per tube, centrifuge (5 min.) at 200 - 300 x *g*, and discard supernatant.
2. **Fix and permeabilize cells with BD Cytofix/Cytoperm Buffer.**
   a. Resuspend cells with 100 µL of BD Cytofix/Cytoperm Buffer per tube.
   b. Incubate cells for 15 - 30 minutes at room temperature or on ice.
   c. Wash cells 1x with 1 mL of 1x BD Perm/Wash Buffer, centrifuge as in step 1d and discard supernatant.

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3. **Incubate cells with BD Cytoperm™ Permeabilization Buffer Plus.**
   a. Resuspend cells with 100 µL of BD Cytoperm™ Permeabilization Buffer Plus per tube.
   b. Incubate cells for 10 minutes on ice.
   c. Wash cells 1x by adding 1 mL of 1x BD Perm/Wash Buffer (as in Step 2c).
4. **Re-Fixation of cells**
   a. Resuspend cells with 100 µL of BD Cytofix/Cytoperm Buffer per tube.
   b. Incubate cells for 5 minutes at room temperature or on ice.
   c. Wash cells 1x by adding 1 mL of 1x BD Perm/Wash Buffer (as in Step 2c).
5. **Treatment of cells with DNase to expose incorporated BrdU.**
   a. Resuspend cells with 100 µL of diluted DNase (diluted to 300 µg/mL in DPBS) per tube, (ie, 30 µg of DNase to each tube).
   b. Incubate cells for 1 hour at 37°C.
   c. Wash cells 1x by adding 1 mL of 1x BD Perm/Wash Buffer (as in Step 2c).
6. **Stain BrdU and intracellular antigens with fluorescent antibodies.**
   a. Resuspend cells with 50 µL of BD Perm/Wash Buffer containing diluted fluorescent anti-BrdU and/or antibodies specific for intracellular antigens.
   b. Incubate cells for 20 minutes at room temperature.
   c. Wash cells 1x by adding 1 mL of 1x BD Perm/Wash Buffer (as in Step 2c).
7. **Optional - Staining of total DNA for cell cycle analysis.**
   Note: Proceed to Step 8 if the staining of total DNA levels is not desired.
   a. Resuspend cells with 20 µL of the 7-AAD solution (Cat. No. 559925).
8. **Resuspension of cells for Flow Cytometric Analysis.**
   a. Add 1 mL of staining buffer to each tube to resuspend cells.
   b. Analyze stained cells with a flow cytometer (run at a rate no greater than 400 events/sec.) and acquire multiparameter data files.
   Note: Samples may be stored overnight at 4°C, protected from exposure to light, prior to analysis by flow cytometry.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 mL	(none)
554722	Fixation and Permeabilization Solution	125 mL	(none)
554723	Perm/Wash Buffer	100 mL	(none)
550891	Bromodeoxyuridine (BrdU)	25 mg	(none)
556028	FITC Mouse Anti- BrdU Set	100 Tests	(none)
555627	Purified Mouse Anti- BrdU	0.1 mg	3D4
559925	7-AAD	2 mL	(none)

Product Notices

1. Cy is a trademark of Amersham Biosciences Limited.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

BD Pharmingen™. *BrdU Flow Kits Instruction Manual.* San Jose, CA: BD Biosciences; 2008:1-40. (Methodology)

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