

Technical Data Sheet

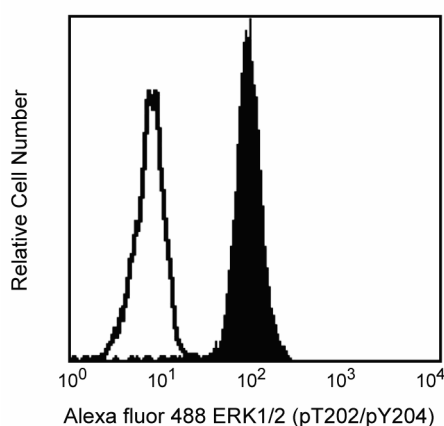
Perm Buffer II

Product Information

Material Number:	558052
Size:	125 mL
Storage Buffer:	Aqueous buffered solution containing proprietary ingredients.

Description

BD Phosflow™ Perm Buffer II is intended to be used for the permeabilization of fixed PBMC's or lysed whole blood. PBMC's, for example, can be stimulated with kinase activators, such as phorbol esters, and can be fixed with BD Phosflow™ Fix Buffer I (Cat. No. 557870) followed by permeabilization of the cells with BD Phosflow™ Perm Buffer II to allow for an effective intracellular staining of kinases. Designed for optimal fluorescent staining in flow cytometry, BD Phosflow™ Perm Buffer II can be used with antibodies for phospho-protein analysis (i.e the staining of intracellular proteins involved in cell signalling pathways, such as phosphorylated proteins and assorted kinases). In many cases, these phospho-specific antibodies may be used simultaneously with the staining of cell surface markers (e.g CD markers). Not all cell surface markers, however, are compatible with BD Phosflow™ Perm Buffer II. For a current listing of compatible cell surface markers for use with BD Phosflow™ Perm Buffer II, investigators are encouraged to visit the following website:
http://www.bdbiosciences.com/documents/antibodies_human_cellsurface_marker.pdf.



Flow cytometric analysis of ERK1/2 (pT202/pY204) in human PBMC. Freshly isolated human PBMC were either left untreated (unshaded histogram) or treated (shaded histogram) with 40 nM PMA at 37°C for 10 minutes. The PBMC were then fixed using BD Phosflow™ Fix Buffer I (Cat. No. 557870) and permeabilized with BD Phosflow™ Perm Buffer II for 30 minutes on ice. After washing the cells twice with BD Pharmingen™ Stain Buffer (Cat. No. 554656), the PBMC were stained with Alexa Fluor® 488 Rat Anti-ERK1/2 (pT202/pY204) (Cat. No. 612592).

Preparation and Storage

Store undiluted at room temperature.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
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Recommended Assay Procedure:

Flow cytometry:

1. Warm BD Phosflow™ Fix Buffer I (Cat. No. 557870) to 37 °C prior to use.
2. Chill BD Phosflow™ Perm Buffer II to -20 °C prior to use.
3. Collect PBMCs after Ficoll-Paque separation and treat with any appropriate cell activators as applicable.
4. At the end of the treatment, immediately mix one volume of the warmed BD Phosflow™ Fix Buffer I with one volume of the PBMC suspension. Mix well and incubate the tubes in a 37 °C water bath for 10 min. Spin down the cells at 250 x g for 10 min in a table-top centrifuge and then aspirate the supernatant.
5. Wash the cells once with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656). Alternatively, 1X Washing/Staining Solution (1 x PBS, 1% FCS, and 0.09% sodium azide) may be used. Centrifuge to pellet the cells and remove the supernatant.
6. Vortex to loosen the cells. Permeabilize the cells by slowly adding cold BD Phosflow™ Perm Buffer II while vortexing. Incubate on ice for 30 min and then spin down the cells.
7. Wash the cells twice with 5-10 mL of BD Pharmingen™ Stain Buffer (FBS). Centrifuge at 250 x g for 10 minutes and remove the supernatant by aspiration.
8. Resuspend cells in BD Pharmingen™ Stain Buffer (FBS) at 1x 10⁷/ml and aliquot 100 µl to each flow tube to continue with antibody staining and flow cytometric analysis.

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Danger: BD Phosflow™ Perm Buffer II contains 64.86% methanol (w/w).

Hazard statements

Highly flammable liquid and vapor.

Toxic if swallowed, in contact with skin or if inhaled.

Causes damage to the central nervous system. Route of exposure: Oral.

Precautionary statements

Keep away from heat/sparks/open flames/hot surfaces. - No smoking.

Wear protective clothing / eye protection.

Wear protective gloves.

Do not breathe mist/vapours/spray.

IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
557870	Fix Buffer I	250 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
612592	Alexa Fluor® 488 Mouse Anti-ERK1/2 (pT202/pY204)	50 Tests	20A
554655	Fixation Buffer	100 mL	(none)
558049	Lyse/Fix Buffer 5X	250 mL	(none)
557885	Perm/Wash Buffer I	125 mL	(none)
558050	Perm Buffer III	125 mL	(none)

Product Notices

1. Ficoll-Paque is a trademark of Amersham Biosciences Limited.
2. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
4. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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