

## Technical Data Sheet

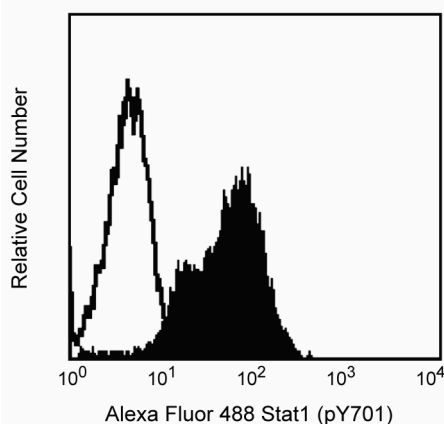
## Perm Buffer III

## Product Information

**Material Number:** 558050  
**Size:** 125 mL  
**Storage Buffer:** Aqueous buffered solution containing proprietary ingredients.

## Description

BD Phosflow™ Perm Buffer III is intended to be used for the permeabilization of fixed PBMCs or lysed whole blood. PBMCs, 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 III to allow for an effective intracellular staining of kinases. Designed for optimal fluorescent staining in flow cytometry, BD Phosflow™ Perm Buffer III 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 III. For a current listing of compatible cell surface markers for use with BD Phosflow™ Perm Buffer III, investigators are encouraged to visit the following website:  
[http://www.bdbiosciences.com/documents/antibodies\\_human\\_cellsurface\\_marker.pdf](http://www.bdbiosciences.com/documents/antibodies_human_cellsurface_marker.pdf).



**Flow cytometric analysis of Stat1 (pY701) in human PBMC.** Freshly isolated human PBMC were either left untreated (unshaded histogram) or treated (shaded histogram) with 10,000 units of interferon- $\alpha$  at 37°C for 10 to 15 minutes. The PBMC were then fixed using BD Phosflow™ Fix Buffer I (Cat. No. 557870) and permeabilized with BD Phosflow™ Perm Buffer III 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 Mouse Anti-Stat1 (pY701) (Cat. No. 612596).

## Preparation and Storage

Store undiluted at room temperature.

## Application Notes

## Application

|   |                  |
|---|------------------|
| Intracellular staining (flow cytometry) | Routinely Tested |
|---|------------------|

## 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 III 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 III 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 10<sup>7</sup>/ml and aliquot 100  $\mu$ l to each flow tube to continue with antibody staining and flow cytometric analysis.

## BD Biosciences

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558050 Rev. 4



**Danger:** BD Phosflow™ Perm Buffer III contains 87.68% 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**

Wear protective clothing / eye protection.

Wear protective gloves.

Take off immediately all contaminated clothing. Wash contaminated clothing before reuse.

Wash thoroughly after handling.

Use only outdoors or in a well-ventilated area.

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

Ground and bond container and receiving equipment. Use explosion-proof [electrical/ventilating/lighting/] equipment.

Use non-sparking tools. Take action to prevent static discharges.

Do not breathe mist/vapours/spray.

Do not eat, drink or smoke when using this product.

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.

IF SWALLOWED: Immediately call a POISON CENTER/doctor. Rinse mouth.

If exposed: Call a poison center/doctor.

In case of fire: Use suitable extinguishing media to extinguish.

Store in well-ventilated place. Keep cool. Store locked up. Keep container tightly closed.

#### **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)       |
| 612596                | Alexa Fluor® 488 Mouse Anti-Stat1 (pY701) | 50 Tests    | 4a           |
| 554655                | Fixation Buffer                           | 100 mL      | (none)       |
| 558049                | Lyse/Fix Buffer 5X                        | 250 mL      | (none)       |
| 557885                | Perm/Wash Buffer I                        | 125 mL      | (none)       |
| 558052                | Perm Buffer II                            | 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](http://www.bdbiosciences.com/colors).
4. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://www.bdbiosciences.com/pharmingen/protocols) for technical protocols.