Technical Data Sheet

Brilliant Stain Buffer

Product Information

Material Number:	**566349**
Size:	1000 Tests
Vol. per Test:	50 µl/test
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The BD Horizon™ Brilliant Stain Buffer is a buffer for the immunofluorescent staining of cells. Brilliant Stain Buffer is a solution that is added to mixtures of certain fluorescent reagents before staining cells. It was designed to complement multicolor flow cytometry experiments that utilize two or more different staining reagents conjugated with BD Horizon Brilliant fluorescent polymer dyes. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. For optimal and reproducible results, BD Horizon Brilliant Stain Buffer should be used anytime two or more BD Horizon Brilliant dyes are used in the same experiment. When using only one BD Horizon Brilliant reagent in an experiment, the Brilliant Stain Buffer is not needed. The BD Horizon Brilliant Stain Buffer is compatible with the use of other fluorescent staining reagents conjugated with traditional fluorochromes, such as fluorescein, phycoerythrin or Alexa Fluor® dyes.

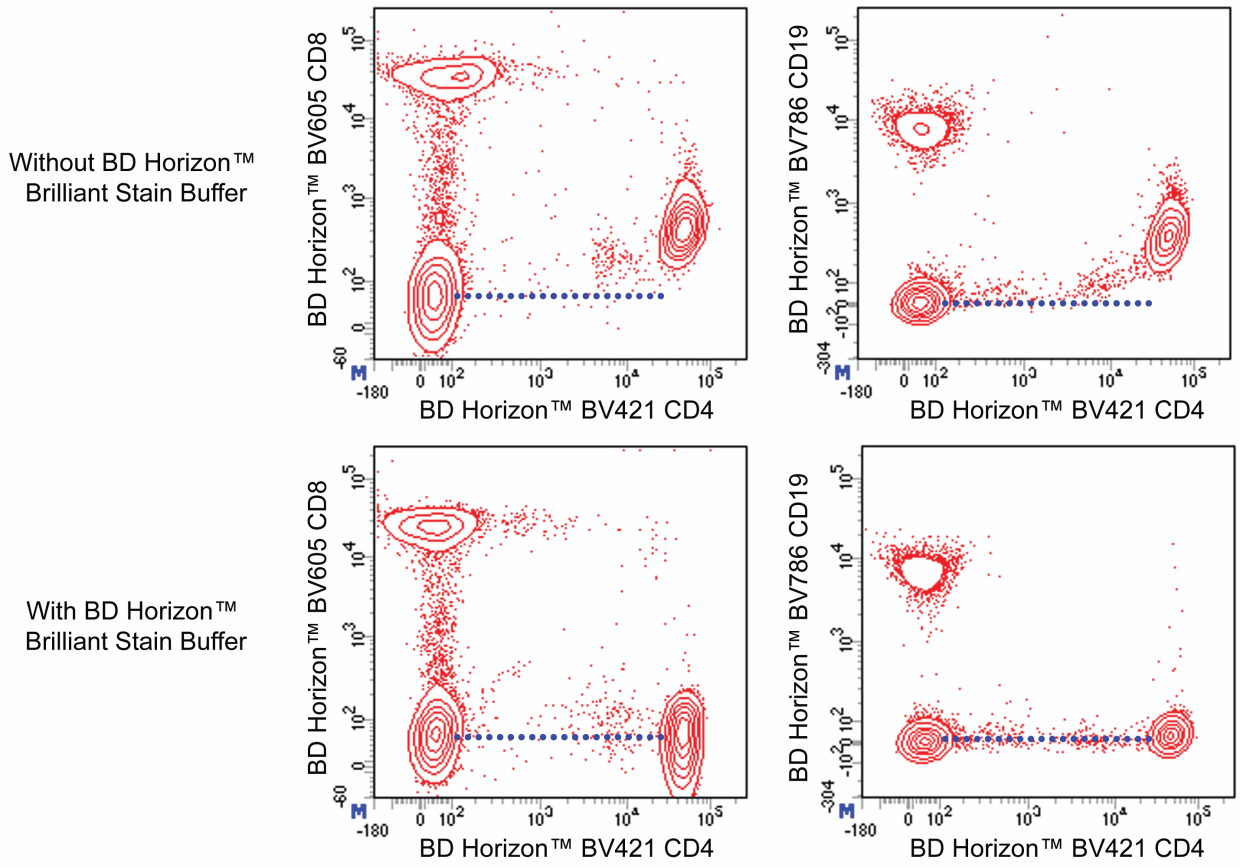


Figure 1. BD Horizon Brilliant Stain Buffer should be used in multicolor immunofluorescent staining and flow cytometric analysis when using two or more BD Horizon Brilliant reagents. *Whole human blood was stained with BD Horizon™ BV421 Mouse Anti-Human CD4 antibody and either BD Horizon™ BV605 Mouse Anti-Human CD8 antibody (Left Panels) or BD Horizon™ BV786 Mouse Anti-Human CD19 antibody (Right Panel). Erythrocytes were lysed with BD FACS Lysing Solution (Cat. No. 349202). Two-color flow cytometric dot plots show the correlated expression patterns of CD4 versus either CD8 or CD19 for gated events with the forward and side light-scatter characteristics of viable lymphocytes. Staining cells in the presence of BD Horizon™ Brilliant Stain Buffer (Cat. No. 563794/566349; Lower Panels) restores the major lymphocyte populations to their expected fluorescent staining patterns (dotted lines) when compared with cells stained without Brilliant Stain Buffer (Upper Panels). Flow cytometric analysis was performed using a BD™ LSR II Flow Cytometer System.*

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Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Recommended Assay Procedure:

Protocols for Multicolor Immunofluorescent Staining of Cells Using BD Horizon Brilliant Stain Buffer

Multicolor Staining of Human Cell Samples in Tubes or 96-Well Plates Using Individual Staining Reagents

1. Add 50 µL of BD Horizon Brilliant Stain Buffer to all tubes or desired wells for the experiment

   Note: The 50 µL amount of Brilliant Stain Buffer per tube or per well does not depend on the final staining volume or amount of cells used per tube or number of BD fluorescent antibodies used in staining. Although written for use with human cells, these protocols can readily be adapted for analyzing mouse cells or cells from other species, for example, by staining mouse cells at 4°C rather than at room temperature (RT).

2. Add each fluorescent reagent at the recommended volume per test (eg, 5 µL or 20 µL) and then proceed to either Protocol 1, 2, or 3.

Protocol 1 for Staining Whole Blood Samples in Tubes

a. Add 100 µL of human whole blood to each tube
b. Vortex tube contents
c. Incubate (30 min) the suspended cells protected from light at room temperature (RT)
d. Add 2 mL of BD FACS™ Lysing Solution (Cat. No. 349202; 10 min) or BD Pharm Lyse™ Lysing Buffer (Cat. No. 555899; 15 min) per tube and incubate protected from light at RT
e. Pellet cells by centrifugation (5 min) at 1400-1500 rpm
f. Aspirate supernatant; add 2-3 mL of stain/wash buffer, eg, BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) or BD Pharmingen™ Stain Buffer (BSA) (Cat. No. 554657)
g. Pellet cells by centrifugation (5 min) at 1400-1500 rpm
h. Aspirate the supernatant and resuspend cells in 500 µL of stain/wash buffer for flow cytometric analysis

Protocol 2 for Staining Peripheral Blood Mononuclear Cells or Bulk Erythrocyte-lysed Whole Blood Samples in Tubes

a. Add 100 µL of human cells to each tube
b. Vortex tube contents
c. Incubate (30 min) the suspended cells protected from light at room temperature (RT)
d. Add 2 ml of stain/wash buffer per tube
e. Pellet cells by centrifugation (5 min) at 1400-1500 rpm
f. Aspirate supernatant; add 2-3 mL of stain/wash buffer
g. Pellet cells by centrifugation (5 min) at 1400-1500 rpm
h. Aspirate the supernatant and resuspend cells in 500 µL of stain/wash buffer for flow cytometric analysis

Protocol 3 for Staining Peripheral Blood Mononuclear Cells or Bulk Erythrocyte-lysed Whole Blood Samples in 96-well Plates

Note: When planning for staining in plates, the user must take into account the volume of the BD Horizon Brilliant Stain Buffer used. Although written for use with human cells, this 96-well plate-based protocol can readily be adapted for analyzing mouse cells or cells from other species.

a. Add 50 µL of human cells to each well
b. Incubate (30 min) protected from light at RT
c. Wash by adding 100 µL of stain/wash buffer
d. Pellet cells by centrifugation (5 min) at 1400-1500 rpm
e. Aspirate supernatants
f. Resuspend pelleted cells by adding 250 µL of stain/wash buffer
g. Pellet cells by centrifugation (5 min) at 1400-1500 rpm
h. Aspirate supernatants
i. Resuspend pelleted cells thoroughly with 150 µL stain/wash buffer by pipetting the suspended cells several times
j. Transfer well contents to tubes and add additional stain/wash buffer to the tubes as desired for flow cytometric analysis

Note: Alternatively, acquire samples for flow cytometric analysis from the plate directly

Multicolor Staining of Human Cell Samples in Tubes or 96-Well Plates Using Cocktailed Staining Reagents

Instead of adding staining reagents individually to each tube or well of a 96-well plate, it may be desirable to add cocktailed staining reagents, ie, mixtures of two or more fluorescent staining reagents. The following protocol provides an example of how to prepare a "per test" 5-Color Fluorescent Antibody Cocktail that already contains BD Horizon Brilliant Stain Buffer.

Human Samples: Pre-mixed Fluorescent Reagent Cocktails

For each multicolor test of cocktailed fluorescent reagents:

i) Add 50 µL of BD Horizon Brilliant Stain Buffer per test
ii) Add each fluorescent reagent at the recommended volume per test (5 µL or 20 µL)
iii) Mix reagents (especially after adding BD Horizon Brilliant reagents)
iv) Store cocktail at 4°C protected from light if it is to be used later

Note: Protected from light, fluorescent reagent cocktails containing more than one Brilliant Violet and/or Brilliant Blue reagent are best used within 24 hours after preparation when stored at 4ºC or within 4 hours when stored at room temperature. However, when more than one Brilliant Ultraviolet (BUV) reagent is in the cocktail, it is best used within 2 hours after preparation irrespective of storage temperature.

Example of creating a 5-Color Fluorescent Antibody Cocktail containing 2 different Brilliant Violet™ Conjugates
Final Volume per Test = 90 µL

		Total Number of Tests			
	Volume/Test (µL)	1	3	5	10
Brilliant Stain Buffer	50	50	150	250	500
Reagent 1 (BV)	5	5	15	25	50
Reagent 2 (BV)	5	5	15	25	50
Reagent 3	5	5	15	25	50
Reagent 4	5	5	15	25	50
Reagent 5	20	20	60	100	200
Total Volume	**90**	**90**	**270**	**450**	**900**

Add desired volume of Reagent Cocktail (90 µL in this 5-color example) to all tubes or wells using the protocols for staining human cells described above.

Compensation and Setup

BD Horizon Brilliant Stain Buffer can be used in single color compensation controls using cells. The buffer is compatible with BD™ Compbeads, however, it has not been tested with compensation beads from other vendors.

Suggested Companion Products

Catalog Number	Name	Size	Clone
563794	Brilliant Stain Buffer	100 Tests	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
554657	Stain Buffer (BSA)	500 mL	(none)
349202	BD FACS™ Lysing Solution	100 mL	(none)
555899	Lysing Buffer	100 mL	(none)
566385	Brilliant Stain Buffer Plus	1000 Tests	(none)

Product Notices

1. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
2. 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.
3. Alexa Fluor® is a registered trademark of Life Technologies Corporation.
4. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
5. BD Horizon Brilliant Stain Buffer is covered by one or more of the following US patents: 8,110,673; 8,158,444; 8,575,303; 8,354,239.

References

Acosta JR, Douagi I, Andersson DP, et al. Increased fat cell size: a major phenotype of subcutaneous white adipose tissue in non-obese individuals with type 2 diabetes. *Diabetologia.* 2016; 59(3):560-70. (Methodology: Flow cytometry)
Andrés-Blasco I, Herrero-Cervera A, Vinué Á, et al. Hepatic lipase deficiency produces glucose intolerance, inflammation and hepatic steatosis. *J Endocrinol.* 2015; 227(3):179-91. (Methodology: Flow cytometry)

Ingelman-Sundberg HM, Laestadius Å, Chrapkowska C, et al. Diverse effects on vaccine-specific serum IgG titres and memory B cells upon methotrexate and anti-TNF-α therapy in children with rheumatic diseases: A cross-sectional study. *Vaccine.* 2016; 34(10):1304-11. (Methodology: Flow cytometry)
Leijten EF, van Kempen TS, Boes M, et al. Brief report: enrichment of activated group 3 innate lymphoid cells in psoriatic arthritis synovial fluid. 2015; 67(10):2673-8. (Methodology: Flow cytometry)
McKay FC, Gatt PN, Fewings N, et al. The low EOMES/TBX21 molecular phenotype in multiple sclerosis reflects CD56+ cell dysregulation and is affected by immunomodulatory therapies. *Clin Immunol.* 2016; 163:96-107. (Methodology: Flow cytometry)
O'Connor KS, Read SA, Wang M, et al. IFNL3/4 genotype is associated with altered immune cell populations in peripheral blood in chronic hepatitis C infection. *Genes Immun.* 2016; 17(6):328-34. (Methodology: Flow cytometry)
Vinué Á, Andrés-Blasco I, Herrero-Cervera A, et al. Ink4/Arf locus restores glucose tolerance and insulin sensitivity by reducing hepatic steatosis and inflammation in mice with impaired IRS2-dependent signalling. *Biochim Biophys Acta.* 2015; 1852(9):1729-42. (Methodology: Flow cytometry)