Technical Data Sheet

Stain Buffer (BSA)

Product Information

Material Number: **554657**

Size: 500 ml

Description

PharmingenStain Buffer (BSA) can be used for the immunofluorescent staining of single-cell suspensions prepared from either lymphoid tissues, bone marrow, peripheral blood, or cultured cells. PharmingenStain Buffer (BSA) is useful for the dilution and application of fluorescent reagents as well as for the suspension, washing, and storage of cells destined for flow cytometric analysis (or fluorescence microscopy). Based on previous descriptions of staining media, PharmingenStain Buffer (BSA) was formulated as a neutral pH (pH 7.4) -buffered salt solution (*i.e.,* DPBS) that is supplemented with 0.2% (w/v) bovine serum albumin (BSA) proteins. As such, PharmingenStain Buffer (BSA) is designed to maintain cell viability and maximize fluorescence signal intensities generated by pH-sensitive fluorochromes, *e.g.,* fluoresceine isothiocyanate (FITC) . PharmingenStain Buffer (BSA) is useful for staining cells with biotinylated antibodies and fluorochrome-conjugated avidins because this staining medium contains no biotin . When present in staining media, free biotin can interfere with the binding of fluorescent-avidins to biotinylated antibodies that have complexed with their cognate, cell-associated antigens. Moreover, free biotin can bind to cells and contribute to an increase in the non-specific, background staining of cells that are exposed to fluorochrome-conjugated avidins. PharmingenStain Buffer (BSA) contains the metabolic inhibitor, sodium azide (NaN3). NaN3 inhibits the potential redistribution of cell surface antigens (*e.g.,* due to shedding or internalization) caused by antibody crosslinking. NaN3 (in combination with maintenance of cold ambient temperatures) thereby prevents the potential loss of fluorescent signal intensities generated by immunofluorescently-stained cells during subsequent flow cytometric analysis(or fluorescence microscopy).

Preparation and Storage

Store undiluted at 4°C.

Application Notes

Application

Flow cytometry	Routinely Tested
Fluorescence microscopy	Tested During Development
Intracellular staining (flow cytometry)	Tested During Development

Recommended Assay Procedure:

Procedure for Using Pharmingen Stain Buffer (BSA) for the Direct Immunofluorescent Staining of Cells.

1. Prepare single-cell suspensions from either lymphoid tissue, bone marrow, peripheral blood or cell cultures using standard protocols.
2. Wash the cells twice in cold Pharmingen Stain Buffer (BSA) and pellet the cells by centrifugation (e.g., 300 x g at 4°C). Resuspend the cell pellet with cold Pharmingen Stain Buffer (BSA) to a final concentration of 2 x 10e7 cells/ml.
3. Distribute 50 µl aliquots of the cell suspension (10e6 cells) to either tubes or the round-bottomed wells of microwell plates.
4. Dilute fluorescent antibodies to their predetermined optimal concentrations in Pharmingen Stain Buffer (BSA) and add small aliquots (e.g., 10 µl) of the diluted antibodies to the tubes or microwells that contain the target cell suspensions. Incubate for 20 minutes on ice protected from light. Staining time may be increased (≥ 45 min) depending on the avidity of the fluorescent antibody.
5. Wash the cells two times with either 200 µl (for microwell plates) or 1 ml (for tubes) volumes of Pharmingen Stain Buffer (BSA) to remove unbound antibodies. Centrifuge cells as 300 x g for 5 min. After each centrifugation, carefully aspirate (for microwell plates or tubes) or invert and blot away (for tubes) supernatants from cell pellets.
6. Resuspend the cell pellet in either 200 µl (for microwell plates) or 0.5 ml (for tubes) volumes of Pharmingen Stain Buffer (BSA). Transfer stained cells from microwell plates to the appropriate tubes for flow cytometric analysis (adjust final volume to ~0.5 ml).
7. Analyze stained cell samples either by flow cytometry or by fluorescence microscopy as soon as possible (e.g., ≤ 4 hours) after staining. If analysis must be delayed, then the stained cells can be fixed with buffered paraformaldehyde (e.g., Cytofix Buffer; Cat. No. 554657) and stored at 4°C (protected from light). The fixed cells should be analyzed as soon as possible (e.g., up to one week after staining and fixation).

Note 1: Pharmingen Stain Buffer (BSA) can similarly be used for the indirect immunofluorescent staining of cells. In this case, repeat steps 4 and 5 when using either unlabeled or biotinylated primary antibodies.

Note 2: Pharmingen Stain Buffer (BSA) can also be used for the immunofluorescent staining of surface antigens expressed by cells that are destined to be fixed and immunofluorescently stained for intracellular antigens such as cytokines (for details see reference #6 or the online protocols at our web site at www.bdbiosciences.com/pharmingen/protocols/). Cells stained for intracellular cytokines can be resuspended and maintained (i.e., at 4°C, protected from light) in Pharmingen Stain Buffer (BSA) prior to analysis by either flow cytometry or fluorescence microscopy

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Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

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