Technical Data Sheet

Stain Buffer (FBS)

Product Information

Material Number:	**554656**
Size:	500 ml
Storage Buffer:	Aqueous buffered solution containing fetal bovine serum and ≤0.09% sodium azide.

Description

Stain Buffer (FBS) can be used for the immunofluorescent staining of single-cell suspensions prepared from either lymphoid tissues, bone marrow, peripheral blood, or cultured cells. Stain Buffer (FBS) 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. Based on previous reports of staining media, Stain Buffer (FBS) was formulated as a neutral pH (pH 7.4)-buffered salt solution (i.e., DPBS) that is supplemented with heat-inactivated (56°C, 30 minutes) fetal bovine serum (FBS) proteins. As such, Stain Buffer (FBS) is designed to maintain cell viability and maximize fluorescence signal intensities generated by pH-sensitive fluorochromes, e.g., fluoresceine isothiocyanate (FITC). In addition, Stain Buffer (FBS) 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.

Preparation and Storage

Store undiluted at 4°C.

Application Notes

Application

Flow cytometry	Routinely Tested

Recommended Assay Procedure:

Flow Cytometry (Direct immunofluorescence staining):

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 Stain Buffer (FBS) and pellet the cells by centrifugation (e.g., 300 x g at 4°C). Resuspend the cell pellet with cold Stain Buffer (FBS) 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 Stain Buffer (FBS) 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 Stain Buffer (FBS) 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 Stain Buffer (FBS). 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 by flow cytometry 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., BD Cytofix™ Fixation Buffer; Cat. No. 554655) 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: Stain Buffer (FBS) 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. When staining cells with biotinylated antibodies and fluorochrome-conjugated avidin, it is desirable to use a staining medium that contains no biotin, such as Stain Buffer (BSA) (Cat. No. 554657).

Note 2: Stain Buffer (FBS) 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. Cells stained for intracellular cytokines can be resuspended and maintained (i.e., at 4°C, protected from light) in Stain Buffer (FBS) prior to analysis by flow cytometry.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.259.0187	32.53.720.550	0120.8555.90	65.6861.0633	55.11.5185.9995

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
554657	Stain Buffer (BSA)	500 ml	(none)

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

Cytokine/Chemokine Manual. Genes to Proteins to Cells. Application Manual. 2nd Edition.. :29-47.(Biology: Flow cytometry)

Holmes K, Fowlkes BJ, Schmid I, Giorgi JV. Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W, ed. *Current Protocols in Immunology*. New York: Greene Publishing Associates/Wiley-Interscience; 1995:5.3.1-5.3.23.(Biology)

Mishell BB, Shiigi SM, Henry C, et al. Mishell BB, Shiigi SM, ed. *Selected Methods in Cellular Immunology*. San Francisco: WH Freeman and Co; 1980:3-27.(Biology)

Jackson AL, Warner NL. Rose NR, Friedman H, Fahey JL, ed. *Manual of Clincial Laboratory Immunology, Third Edition*. Washington DC: American Society for Microbiology; 1986:226-235.(Biology)

Ohkuma S, Poole B. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. *Proc Natl Acad Sci U S A.* 1978; 75(7):3327-3331.(Biology)