Flow Fix 2% Paraformaldehyde Fixative Kit

Catalog Number: 25085

DESCRIPTION

This kit contains the materials required to make a working stock of 2% w/v paraformaldehyde in a neutral-buffered saline solution. This sodium azide-free buffer is designed for fixation of cells prior to downstream flow cytometry applications such as fluorescence-activated cell sorting (FACS) and fluorescence in situ hybridization (FISH-FACS). This buffer can be used for fixation both prior to immunofluorescent staining, as well as following staining in order to preserve cells for downstream analysis. Flow Fix may be useful to avoid the capping or shedding of fluorescent antibodies and/or surface antigens during the period before flow cytometric analysis.

PREPARATION OF 2% FLOW FIX PARAFORMALDEHYDE FIXATIVE

1. Add 250 mL Fixative Solution to the 500 mL bottle provided.
2. Add 250 mL 0.2M Phosphate Buffer to the bottle, replace cap, and then mix gently by inverting/swirling solution.
3. Affix provided label to outside of 500 mL bottle for a final solution of 2% paraformaldehyde in 0.1M phosphate buffer.

Note: The kit materials are not provided sterile, if desired, this solution can be filtered using a sterile 0.22 micron vacuum filter unit in a laminar flow hood. This solution may also be sterilized by autoclave at 121°C for 20 minutes on liquid cycle.

GENERAL PROCEDURE FOR FIXING CELLS FOR FLOW CYTOMETRY

Cells can fixed for downstream immunostaining, or if flow cytometry analysis is unable to be performed on the cells within an hour of collection. Depending on the antigen of interest, some fixation protocol optimization may be required.

Fixation will not retain cell viability, if cells must be analyzed live and then saved for further analysis, delay fixation until after live analysis. If surface and intracellular analysis of the same sample is intended, first complete staining of cell surface antigens before fixing cells to retain surface antigen integrity.

1. Collect and pellet cells by centrifugation and aspirate supernatant.
2. Wash cells with 1X PBS to remove residual medium, centrifuging and aspirating resulting supernatant.
3. Resuspend cells in 4°C 2% Flow Fix at 500 µL per 1.0 x 10^6 cells, ensuring the cells are not clumped prior to incubation.
4. Incubate at room temperature for 15 minutes.
5. Wash cells with 1X PBS to remove fixative solution, discarding supernatant.
6. Resuspend cells according to desired downstream application:
   Cells can be resuspended in 1X PBS and stored at 4°C protected from light, remaining stable for several days post-fixation.
   Preparation can continue with permeabilization procedure and downstream immunostaining.

STABILITY

Prepared mixture is stable for up to 14 days when stored at 4°C and protected from prolonged exposure to light.
Material can be aliquoted and frozen at -20°C, stable for up to 2 years.

MATERIALS SUPPLIED

Catalog Number	Description	Volume
25037A-250	0.2M Phosphate Buffer pH 7.3	250 mL
25085B-250	Fixative Solution	250 mL
	500 mL Square Bottle	
	2% Flow Fix Label	

SAFETY

This product is for R&D use only. Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

REFERENCES

1. Lanier L. & Warner N. (1981) *Paraformaldehyde fixation of hematopoietic cells for quantitative flow cytometry (FACS) analysis.* Journal of Immunological Methods, 47(1): 25-30.
2. Smit, J., Meijer, C., & Decary, F. (1974). *Paraformaldehyde fixation in immunofluorescence and immunoelectron microscopy: Preservation of tissue and cell surface membrane antigens.* Journal of Immunological Methods, 6(1-2): 93-98.

RELATED PRODUCTS

Cat #	Description	Size
25037-1	Flow Fix, 1% Paraformaldehyde Fixative Kit	1 kit

ORDERING INFORMATION

Cat. #	Description	Size
25085-1	Flow Fix, 2% Paraformaldehyde Fixative Kit	1 Kit

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