R&D SYSTEMS
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FlowX™ FoxP3/Transcription Factor Fixation & Permeabilization Buffer Kit

Catalog Number: FC012

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	25-TESTS		100-TESTS		DESCRIPTION	STORAGE OF DILUTED MATERIAL
	PART #	SIZE	PART #	SIZE		
FoxP3 Fixation/ Transcription Factor Concentrate (4X)	894065	8 mL	894733	30 mL	A formaldehyde solution.	Once diluted, components should be used and discarded.
FoxP3 Fixation/ Transcription Factor Diluent	894068	25 mL	894734	100 mL	A buffered detergent.	
FoxP3/ Transcription Factor Permeabilization and Wash Buffer (10X)	894356	25 mL	894735	100 mL	A buffered protein base with detergent and preservatives. **Note:** *May contain a precipitate but will not affect product performance.*	

PRODUCT DESCRIPTION

FlowX™ FoxP3/Transcription Factor Fixation & Permeabilization Buffer Kit is formulated and optimized for immunofluorescent staining of FoxP3$^+$ or other transcription factor positive single-cell suspensions intended for flow cytometric acquisition and analysis. This product is supplemented with the metabolic inhibitor sodium azide.

INTENDED USE

This product is designed for flow cytometry applications with fluorochrome-conjugated antibodies. Refer to product specification sheets to obtain the recommended working antibody dilutions.

STAINING PROTOCOL

1. Wash human PBMCs or mouse splenocytes (1 x 10^6 cells per sample) with 2 mL of Flow Cytometry Staining Buffer (R&D Systems®, Catalog # FC001) or other BSA-containing buffer, by spinning at 300 x g for 5 minutes, using 5 mL flow cytometry tubes.
2. Fc-block cells with blocking IgG (1 µg IgG/10^6 cells) for 10 minutes at 2-8 °C.
3. Surface stain with desired antibody for 30 minutes at 2-8 °C.
4. Wash cells two times with **cold** 1X PBS. During washes, make up fresh 1X FoxP3/Transcription Factor Fixation Buffer by diluting FoxP3/Transcription Factor Fixation Concentrate (4X) with FoxP3/Transcription Factor Fixation Diluent (*ie.* 100 µL FoxP3/Transcription Factor Fixation Concentrate (4X) + 300 µL FoxP3/Transcription Factor Fixation Diluent).
5. Resuspend cells in fresh 1X FoxP3/Transcription Factor Fixation Buffer using 0.5 mL/tube. Incubate at 2-8 °C for 30 minutes. During this incubation, make up 1X FoxP3/Transcription Factor Permeabilization and Wash Buffer by diluting FoxP3/Transcription Factor Permeabilization and Wash Buffer (10X) with distilled water (*ie.* 100 µL FoxP3/Transcription Factor Permeabilization and Wash Buffer (10X) + 900 µL diH_2O) and keep at 2-8 °C.
6. Wash two times with fresh, cold, 1X FoxP3/Transcription Factor Permeabilization and Wash Buffer.
7. Add FoxP3 or other transcription factor antibody to cells and incubate for 30 minutes at 2-8 °C.
8. Wash cells one time with cold 1X FoxP3/Transcription Factor Permeabilization and Wash Buffer.
9. Resuspend cells in Flow Cytometry Staining Buffer and run on a flow cytometer.

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Bio-Techne is a trading name for R&D Systems

PRECAUTIONS

This product contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

This product contains formaldehyde, which is a suspected carcinogen. Avoid contact with skin, eyes, and mucous membranes, and avoid inhaling fumes. In case of contact, wash immediately with water and seek medical advice.

TECHNICAL HINTS

- Many cells express Fc receptors (CD16, CD32, and CD64) that bind the Fc region of IgG and thus confound data interpretation. Pretreatment of cells to be stained with a variety of reagents to block Fc receptor mediated antibody interactions is always recommended. Alternatively, preincubation with excess IgG of either human, mouse or rat origin (1 µg of IgG per 1 x 10^5 cells for 15 minutes at room temperature prior to staining) will reduce unwanted Fc receptor interactions. When performing Fc receptor blocking with excess IgG while using unconjugated antibodies, it is imperative that the IgG for Fc receptor blocking not be of the same species of origin as the primary antibody to prevent the secondary antibody from recognizing any Fc receptor bound IgG.
- Setting up control reactions with appropriate isotype controls is useful in data interpretation; however, investigators should realize that different cells might interact to different degrees with isotype control reagents. Matching of immunoglobulin class and isotype, although widely used as a control reaction, may not be suitable in all staining reactions. In some cases comparing the reactivity of the primary antibody between known positive and known negative cells is an acceptable alternative to the use of isotype control reagents.