Technical Data Sheet

Human FoxP3 Buffer Set

Product Information

Material Number:	**560098**
Size:	100 Tests
Component:	**51-9005451**
Description:	Human FoxP3 Buffer A (10 X)
Size:	100 Tests (1 ea)
Component:	**51-9005450**
Description:	Human FoxP3 Buffer B (50 X)
Size:	100 Tests (1 ea)

Description

The Human FoxP3 Buffer Set (Cat. No. 560098) is optimized for use with the FoxP3 mAb clone 259D/C7. It is intented for the fixation and permeabilization of PBMC or lysed whole blood for intracellular staining of Human FoxP3 and surface staining of appropriate CD markers.

Preparation and Storage

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

Irritating to eyes and skin. Do not breathe vapor. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

Application Notes

Recommended Assay Procedure:

Preparation of Buffers Before Use (Working solutions for Human FoxP3 Buffers A and C need to be made fresh for each experimental set)

1. Dilute FoxP3 Buffer A (10X concentrate) 1:10 with room temperature (20°C to 25°C) deionized water.
2. To make a working solution of Buffer C, dilute FoxP3 Buffer B (50X) into 1X FoxP3 Buffer A at a ratio of 1:50 (Buffer B:Buffer A).

Cell Preparation and Staining Procedure for Purified Anti-Human FoxP3 Antibody

1. Bring the buffers to room temperature before use. Prepare working solutions of the Human FoxP3 Buffer Set (Cat. No. 560098) as described in the section above (Preparation of Buffers Before Use).
2. Prepare human PBMC. Calculate needed cells per test. Approximately 1.2 X 10^6 PBMC per test is recommended.
3. Fix cells in bulk or test size, use 2 mL of 1X working solution Human FoxP3 Buffer A per test, incubate for 10 minutes at RT, protected from light. Note: Fixed PBMC in bulk may be frozen at -80°C for up to 24 hours before proceeding to step 4.
4. Wash cells with 2 mL of BD Pharmingen™ Stain Buffer (FBS)* per test. Centrifuge 500 x g for 10 minutes and remove wash buffer.
5. To permeabilize cells in bulk or test size, add 0.5 ml of 1X working solution Human FoxP3 Buffer C per test, incubate for 30 minutes, protected from light.
6. Add an additional 2 mL of Stain Buffer (FBS)* per test. Centrifuge 500 x g for 10 minutes and remove wash buffer.
7. Re-suspend the cells in Stain Buffer (FBS)* to ~1 X 10^7 cells/mL, aliquot 100 µL of cell suspension per 12 x 75 mm tube.
8. Add purified anti-human FoxP3 mAb at appropriate concentrations at 20 µl/test into the tubes. Gently shake or vortex. Incubate for 30 minutes at room temperature, protected from light.
9. Wash the cells twice by adding 2 mL of Stain Buffer (FBS)* to each tube and centrifuge 500 x g for 5 minutes at RT. Remove wash buffer.
10. Add secondary antibody (APC Rat Anti-Mouse IgG1, Cat. No. 550874) at appropriate concentration. Incubate for 30 minutes at room temperature, protected from light.
11. Repeat wash as in Step. 9.
12. Block secondary with normal mouse serum 1:10 in 1 X PBS, 100 µL per test. Incubate for 10 minutes at RT.
13. Add test volumes of anti-human surface mAbs, incubate for 20 minutes at room temperature, protected from light.
14. Add 2 mL of Stain Buffer (FBS)* to each tube and centrifuge 500 x g for 5 minutes at room temperture and remove wash buffer.
15. Re-suspend in wash buffer and analyze immediately. Acquire at least 15,000 to 25,000 CD4 positive lymphocytes.
    Optional: Add 300µl of 1% formaldehyde in 1X PBS and store at 4°C. Analyze cells within 24 hours.

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Cell Preparation and Staining Procedure for Conjugated Anti-Human FoxP3 Antibody

1. Bring the buffers to room temperature before use. Prepare working solutions of the Human FoxP3 Buffer Set (Cat. No. 560098) as described in the section above (Preparation of Buffers Before Use).
2. Prepare human PBMC. Dilute the cells with BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to 1X10^7 cells/mL.
3. Pipette appropriate amount of surface staining reagent to bottom of each 12 x 75 mm tube.
4. Add 100 µL of cells per tube, vortex, incubate for 20 minutes at room temperature, protected from light.
5. Add 2 mL of Stain buffer (FBS)* to wash. Centrifuge 250 x g for 10 minutes, and remove wash buffer.
6. To fix the cells, gently re-suspend pellet in residual volume of wash buffer and then add 2 mL of 1x Human FoxP3 Buffer A. Vortex. Incubate for 10 minutes at room temperature, protected from light.
7. Centrifuge 500 x g for 5 minutes, and remove fixative. **Caution: the pellet is buoyant.**
8. To wash cells, re-suspend each pellet in 2ml of Stain Buffer (FBS)*, and centrifuge 500 x g for 5 minutes. Remove wash buffer.
9. To permeabilize the cells, gently re-suspend pellet in residual volume of wash buffer and then add 0.5 mL of 1X working solution Human FoxP3 Buffer C to each tube. Vortex. Incubate for 30 minutes at room temperature, protected from light.
10. To wash cells, add 2 mL of BD Pharmingen Stain Buffer (FBS)* to each tube, centrifuge 500 x g for 5 minutes at room temperature. Remove buffer and repeat wash step. Remove buffer.
11. Add conjugated FoxP3 antibody at appropriate concentrations to re-suspend the pellet. Gently shake or vortex.
12. Incubate for 30 minutes in the dark at room temperature.
13. Repeat wash as in Step 10.
14. Resuspend in wash buffer and analyze immediately. Acquire at least 15,000 to 25,000 CD4 positive lymphocytes. Optional Add 300 µL of 1% formaldehyde in 1x PBS and store at 4°C. Analyze cells within 24 hours.

* Using BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) is recommended for initial surface staining and all wash steps while covering tubes during incubation steps with caps or parafilm. Optimizing forward scatter and side scatter voltages is also recommended to visualize lymphocytes as separate from debris, red cell ghosts and/or platelets before acquisition.

Warnings and Precautions:

Danger: Human FoxP3 Buffer A (10X) (component 51-9005451) contains 31.05% diethylene glycol (w/w), 10.08% formaldehyde (w/w) and 3.54% methanol (w/w).

Hazard statements:
Harmful if swallowed.
Toxic in contact with skin or if inhaled.
Causes skin irritation.
Causes serious eye damage.
May cause an allergic skin reaction.
Suspected of causing genetic defects.
May cause cancer. Route of exposure: Inhalative.
May cause damage to organs. May cause respiratory irritation.
May cause damage to the kidneys through prolonged or repeated exposure. Route of exposure: Oral.

Precautionary statements:
Wear protective clothing / face protection.
Wear protective gloves.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
IF exposed or concerned: Get medical advice/attention.
If skin irritation occurs: Get medical advice/attention.

Human FoxP3 Buffer B (component 51-9005450) contains ≤ 0.09% sodium azide.

Suggested Companion Products

Catalog Number	Name	Size	Clone
560044	Purified Mouse anti-Human FoxP3	0.1 mg	259D/C7
560045	Alexa Fluor® 647 Mouse anti-Human FoxP3	100 Tests	259D/C7
560046	PE Mouse anti-Human FoxP3	100 Tests	259D/C7
560047	Alexa Fluor® 488 Mouse anti-Human FoxP3	100 Tests	259D/C7
555899	Lysing Buffer	100 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)
550874	APC Rat Anti-Mouse IgG1	0.1 mg	X56

Product Notices

1. 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.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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