

## Technical Data Sheet

## Human FoxP3 Buffer Set

## Product Information

<b>Material Number:</b>	<b>560098</b>
<b>Size:</b>	100 tests
<b>Component:</b>	<b>51-9005451</b>
<b>Description:</b>	Human FoxP3 Buffer A (10 X)
<b>Size:</b>	100 tests (1 ea)
<b>Vol. per Test:</b>	N/A
<b>Component:</b>	<b>51-9005450</b>
<b>Description:</b>	Human FoxP3 Buffer B (50 X)
<b>Size:</b>	100 tests (1 ea)
<b>Vol. per Test:</b>	10 µl

## Description

Human FoxP3 Buffer Set is optimized for use with the FoxP3 mAb clone 259D/C7. It is intended for the fixation and permeabilization of PBMCs (or lysed wholeblood) for intracellular staining of Human FoxP3 and surface staining of the appropriate CD markers. See recommended assay procedure for the Human FoxP3 Buffer Set and the FoxP3 conjugates for the proper use and handling of these products.

## Warnings and Precautions:

Reagent FoxP3 Buffer A contains diethylene glycol and formaldehyde and is a harmful solution.

R20/21/22	Harmful by inhalation, in contact with skin and if swallowed.
R36/37/38	Irritating to eyes, respiratory system and skin.
R40	Limited evidence of a carcinogenic effect.
R43	May cause sensitization by skin contact.
S3	Keep in cool place.
S9	Keep container in a well-ventilated place.
S23	Do not breathe gas/fumes/vapour/spray.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
S60	This material and its container must be disposed of as hazardous waste.

Reagent FoxP3 Buffer B contains ≤0.09% sodium azide.

## Preparation and Storage

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.

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

## Application Notes

## Recommended Assay Procedure:

## Preparation of Buffers Before Use.

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 into 1X FoxP3 Buffer A at a ratio of 1:50 (Buffer B:Buffer A).

\*The working solutions for Human FoxP3 Buffers A and C need to be made fresh for each experimental set.

## Cell Preparation and Staining Procedures for Purified Anti-Human FoxP3 Antibody

1. Bring the buffers to room temperature (RT) before use. Prepare working solutions of the BD Pharmingen Human FoxP3 Buffer Set Cat. No. 560098 (For the buffer preparation, please see buffer instructions above for details).

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2. Prepare human PBMC. Calculate needed cells per test, (1.2 million) PBMC.
3. Fix cells in bulk or test size using 2 ml of 1x working solution Human FoxP3 Buffer A per test, incubate for 10 min. at RT protected from light.

*Note: Fixed PBMC in bulk may be frozen at -80°C for 24 hours before proceeding to step 4.*

4. Wash 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 per test of 1x working solution Human FoxP3 Buffer C per test, incubate for 30 minutes protected from light.
6. Add an additional 2 ml of BD Pharmingen Stain Buffer (FBS)\* per test. Centrifuge 500 x g for 10 minutes and remove wash buffer.
7. Re-suspend the cells in BD Pharmingen Stain Buffer (FBS)\* to 100µl/test; aliquot 100µl of cell suspension per 12 x 75 mm tube.
8. Add purified anti-human FoxP3 mAb at appropriate concentrations at 20µl/test into the tubes. Gently shake or vortex. Incubate for 30 minutes at RT protected from light.
9. Wash the cells twice by adding 2 ml of BD Pharmingen 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 RT protected from light.
11. Repeat wash as in step 9.
12. Block secondary with normal mouse serum 1:10 in 1x 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 RT protected from light.
14. Add 2 ml of BD Pharmingen Stain Buffer (FBS)\* to each tube and centrifuge 500 x g for 5 minutes at RT and remove wash buffer.
15. Re-suspend in wash buffer and analyze immediately.

*Optional Add 300µl of 1% formaldehyde in 1x PBS and store at 4°C. Analyze cells within 24 hours.*

#### **Cell Preparation and Staining Procedures for Conjugated Anti-Human FoxP3 Antibody**

1. Bring the buffers to RT before use. Prepare working solutions of the BD Pharmingen Human FoxP3 Buffer Set Cat. No. 560098 (For the buffer preparation, please see buffer instructions above for details).
2. Prepare human PBMC. Dilute the cells with BD Pharmingen Stain Buffer (FBS)\* to ten million cells/ml.
3. Pipette appropriate amount of surface staining reagent to bottom of each 12 x 75 mm tube.
4. Add 100µl of cells per tube, vortex, incubate for 20 minutes at RT protected from light.
5. Add 2 ml of wash buffer. 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 2ml of 1x Human FoxP3 Buffer A. Vortex. Incubate for 10 minutes at RT in the dark.
7. Centrifuge 500 x g for 5 minutes, and remove fixative. Caution: Be aware the pellet is buoyant.
8. To wash cells, re-suspend each pellet in 2ml of BD Pharmingen 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 RT 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 RT. 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 RT.
13. Repeat wash step #10.
14. Resuspend in wash buffer and analyze immediately.

*Optional Add 300µl of 1% formaldehyde in 1x PBS and store at 4°C. Analyze cells within 24 hours.*

\* We recommend using the BD Pharmingen Stain Buffer (FBS; Cat No. 554656) for initial surface staining and all wash steps and covering tubes during incubation steps with caps or parafilm. We also recommend optimizing forward scatter and side scatter voltages to visualize lymphocytes as separate from debris, red cell ghosts and/or platelets before acquisition.

\*\* Acquire at least 15,000 to 25,000 CD4 positive lymphocytes.

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## Suggested Companion Products

<u>Catalog Number</u>	<u>Name</u>	<u>Size</u>	<u>Clone</u>
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

## Product Notices

1. Please refer to [www.bdbiosciences.com/pharmingen/protocols](http://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.

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