

## Foxp3 Transcription Factor Staining Buffer Kits

Store at 2°C to 8°C

Catalog Numbers: A25866A, A25863A, A25864A, A25865A, A24261

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### Product description

Foxp3 Transcription Factor Staining Buffer Kits are specially formulated to fix, permeabilize, and wash cells in order to facilitate intranuclear staining of fluorescently conjugated anti-mouse Foxp3 antibodies in flow cytometry applications. Effective intracellular cytokine staining can also be achieved with this buffer kit.

### Kit contents

Foxp3 Transcription Factor Staining Buffer Kits are available with or without conjugated Foxp3 antibodies, and contain sufficient buffer reagents for performing 200 tests. The Foxp3 Transcription Factor Wash Buffer is also available as a standalone product.

Component	Foxp3 Transcription Factor Staining Buffer Kit				Wash Buffer
	A25866A	A25863A	A25864A	A25865A	A24261
Foxp3 Transcription Factor Fixation and Permeabilization Buffer A	50 mL	50 mL	50 mL	50 mL	—
Foxp3 Transcription Factor Fixation and Permeabilization Buffer B	160 mL	160 mL	160 mL	160 mL	—
Foxp3 Transcription Factor Wash Buffer	100 mL	100 mL	100 mL	100 mL	100 mL
Foxp3 Mouse Anti-Mouse mAb (APC, FITC, or PE Conjugate)	—	25 µg (APC)	25 µg (FITC)	25 µg (PE)	—

**Caution:** Foxp3 Transcription Factor Fixation and Permeabilization Buffer A contains formaldehyde. Wear appropriate protective eyewear, clothing, and gloves to avoid contact with skin and eyes. Foxp3 Transcription Factor Fixation and Permeabilization Buffer B contains sodium azide. Sodium azide is extremely toxic and may react with lead and copper plumbing to form highly explosive metal azides. Properly dispose of solutions containing sodium azide. Wear appropriate protective eyewear, clothing, and gloves.

### Prepare buffers

Each sample requires 1 mL of 1X Fixation and Permeabilization Solution, and 5 mL of 1X Wash Buffer. Scale the volumes proportionally according to the number of samples being analyzed.

**PBS with 1% BSA\*** (components not included in kit)

Component	Volume
Bovine serum albumin (BSA)	0.05 g
Phosphate buffered saline (PBS), pH 7.1–pH 7.4	To 5 mL

**1X Wash Buffer\***

Component	Volume
Foxp3 Transcription Factor Wash Buffer [10X concentrate]	0.5 mL
PBS with 1% BSA	4.5 mL

\* Store at 2°C to 8°C for up to 6 months.

**1X Fixation and Permeabilization Solution**

Component	Volume
Foxp3 Transcription Factor Fixation and Permeabilization Buffer A (4X concentrate)	250 µL
Foxp3 Transcription Factor Fixation and Permeabilization Buffer B	750 µL

### Intracellular staining protocol

Prepare samples for flow cytometry as directed in the instructions below:

**Important:** Samples should be protected from light at all steps.

1. Perform cell surface staining according to your standard protocol.
2. Add 2 mL flow cytometry staining buffer, and vortex briefly to wash cells.
3. Centrifuge sample at 400 × g, and decant supernatant.
4. Add 1 mL of 1X Fixation and Permeabilization Solution to the cells, and vortex briefly.
5. Incubate for 30–60 minutes at room temperature or 4°C.
6. Add 2 mL 1X Wash Buffer, and vortex briefly to wash the cells. Centrifuge the sample and decant the supernatant.
7. Resuspend sample in 100 µL of 1X Wash Buffer.
8. Add antibody specific for intracellular protein according to your standard protocol, and vortex briefly.
9. Incubate sample for 30–60 minutes at 4°C.
10. Add 2 mL 1X Wash Buffer, and vortex briefly to wash the cells. Centrifuge the sample and decant the supernatant.
11. Resuspend in an appropriate volume of flow cytometry staining buffer.
12. Perform flow cytometry analysis.

## Stability

When stored as instructed, expires six months from date of receipt unless otherwise indicated on the Certificate of Analysis.

## Product documentation

To obtain a Certificate of Analysis or Safety Data Sheets (SDSs), visit [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support).

## Important licensing information

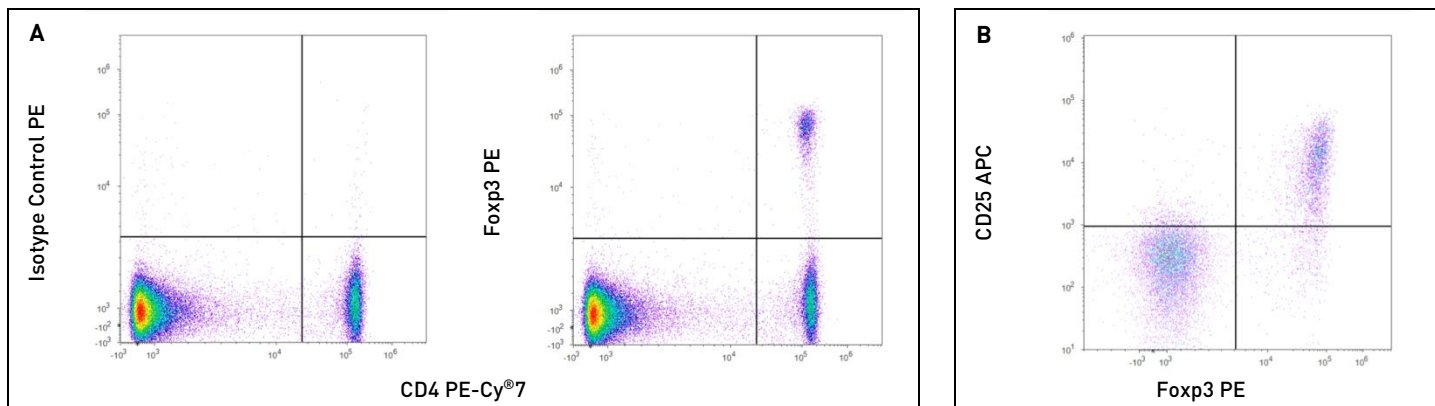
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## Additional products

Product Name	Quantity	Catalog No.
Foxp3 Transcription Factor Wash Buffer	100 mL	A24261
Foxp3 Mouse Anti-Mouse mAb, APC Conjugate	25 µg	A18629
Foxp3 Mouse Anti-Mouse mAb, FITC Conjugate	25 µg	A18662
Foxp3 Mouse Anti-Mouse mAb, PE Conjugate	25 µg	A18690
<b>LIVE/DEAD® Fixable Dead Cell Stain Kits</b>		
Blue (UV excitation)	1 kit (200 assays)	L23105
Violet (405 nm excitation)		L34955
Aqua (405 nm excitation)		L34957
Yellow (405 nm excitation)		L34959
Green (488 nm excitation)		L23101
Red (488 nm excitation)		L23102
Far Red (633/635 nm excitation)		L10120
Near-IR (633/635 nm excitation)		L10119



### Identification of murine regulatory T cells fixed and permeabilized using the Foxp3 Transcription Factor Buffer Kit.

Intracellular staining of C57BL/6 splenocytes surface stained with CD4 and CD25 was performed with a Foxp3 Mouse Anti-Mouse PE mAb or an isotype control. The splenocytes were fixed and permeabilized using the Foxp3 Transcription Factor Buffer Kit to permit intracellular staining, and samples were collected and analyzed using an Attune® Acoustic Focusing Cytometer.

**A.** Specific staining for the Foxp3 transcription factor (right) compared to the isotype control (left). **B.** Assessment of CD25 and Foxp3 expression within the CD4 T cell gate identifies a population of regulatory T cells.

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