FoxP3 Staining Buffer Set

Order no. 130-093-142

Contents

1. Description

This product is for research use only.

Components	**25 mL Fixation/Permeabilization Solution 1**: Four-fold concentrated stock solution for use with Fixation/Permeabilization Solution 2; containing a detergent. **2×40 mL Fixation/Permeabilization Solution 2**: For the dilution of Fixation/Permeabilization Solution 1 prior to use. **40 mL 10× Permeabilization Buffer**: Ten-fold stock solution for dilution prior to use; containing a detergent.
Capacity	100 tests or up to 10^8 total cells.
Storage	Store Fixation/Permeabilization Solution 1 and 2 protected from light at 2–8 °C. Do not freeze. Store 10× Permeabilization Buffer at room temperature. The expiration date is indicated on the vial label.

1.1 Background information

FoxP3, also known as FORKHEAD BOX P3, SCURFIN, and JM2, is a member of the forkhead/winged-helix family of transcriptional regulators. It is expressed predominantly in regulatory T cells (Tregs) and is a major regulator of Treg cell development and function.[1–3]

Mutations in the FoxP3 gene have been linked to the autoimmune manifestations observed in the Scurfy mouse and humans with immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome.[4,5] Studies in mice have shown that FoxP3-deficient animals lack Treg cells, whereas overexpression of the FoxP3 protein leads to profound immune suppression.[3]

The FoxP3 Staining Buffer Set has been developed specifically for use in conjunction with the Anti-FoxP3 antibodies. Failure to use the buffer set and/or different protocols will lead to different or even no results.

1.2 Applications

- Fixation and permeabilization of human, mouse, or non-human primate cells. Optimized for use with clone 3G3 and REA788 Anti-FoxP3 antibodies, human and mouse from Miltenyi Biotec.

For staining protocol refer to
www.miltenyibiotec.com/goto/91a3469fd0fd

2. FoxP3 Staining Buffer Set preparation

▲ Always prepare reagents freshly. Failure to do so may lead to sub-optimal results.

▲ The required total buffer volumes should be calculated beforehand; volumes will depend on the number of cells to be analyzed as well as the number of tests to be performed (for more information, please refer to the Anti-FoxP3 antibodies datasheet).

▲ Caution: The Fixation/Permeabilization Solution 1 contains formaldehyde. Formaldehyde is toxic and a suspected carcinogen; contact with eyes, skin, and mucous membranes should be avoided. Always wear proper protective clothing and gloves when handling the solution.

Fixation and Permeabilization solution
To achieve the appropriate working concentration for safe fixation and permeabilization of cells, the Fixation/Permeabilization Solution 1 must be diluted 1:4 with the Fixation/Permeabilization Solution 2 (i.e. for 10^6 cells use 0.25 mL of Fixation/Permeabilization Solution 1 plus 0.75 mL of Fixation/Permeabilization Solution 2).

Permeabilization Buffer
To achieve the appropriate working concentration for safe permabilization of cells, the 10× Permeabilization Buffer must be diluted 1:10 with deionized or distilled water before use (i.e. 1 mL of 10× Permeabilization Buffer plus 9 mL of deionized/distilled water).

▲ **Note:** Before dilution make sure that buffer does not contain any precipitates.

3. References

1. Hori, S. *et al.* (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299: 1057–1061.
2. Walker, M.R. *et al.* (2003) Induction of FoxP3 and acquisition of T regulatory activity by stimulated human $CD4^+CD25^-$ T cells. J. Clin. Invest. 112: 1437–1443.
3. Ziegler, S.F. (2006) FoxP3: Of Mice and Men. Annu. Rev. Immunol. 24: 209–26.
4. Sakaguchi, S. *et al.* (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J. Immunol. 155: 1151–1164.
5. Lundsgaard, D. *et al.* (2005) *In vivo* control of diabetogenic T-cells by regulatory $CD4^+CD25^+$ T-cells expressing Foxp3. Diabetes 54: 306–310.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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