Store at 4°C

FoxP3/Transcription Factor Fixation/Permeabilization Kit

#43481

1 Kit

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

New 12/18

For Research Use Only. Not For Use In Diagnostic Procedures.

Applications	Species Cross-Reactivity*
F	All

Products Included	Product #	Volume	Storage Temp
FoxP3/Transcription Factor Fixation/Permeabilization Concentrate (4X)	44931	50 ml	4°C
FoxP3/Transcription Factor Fixation/Permeabilization Diluent (1X)	58766	160 ml	4°C
FoxP3/Transcription Factor Permeabilization Buffer (10X)	68751	150 ml	4°C

Description: This kit provides the reagents needed to support antibody-based detection of FoxP3 and other transcription factors by flow cytometry. Optimally formulated fixation and permeabilization buffers halt biological activity and enable antibody access to intracellular targets. Antibody dilution, incubation, and wash steps are performed with the included FoxP3/Transcription Factor Permeabilization Buffer, eliminating the need for separate reagents.

Note: Precipitation may occur in some kit components. The presence of precipitate does not affect the performance of the reagent.

Storage: Store at 4°C. All components in this kit are stable for at least 6 months when stored at 4°C.

Reagents not supplied:

1. Red blood cell lysis buffer
2. Unconjugated or conjugated primary antibody
3. Fluorochrome-conjugated secondary antibody (if applicable)

NOTE: When using any primary or secondary antibody for the first time, titrate the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

4. Cellular dyes (e.g., live-dead discrimination, DNA dyes, etc.), optional

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Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity: H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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FoxP3/Transcription Factor Fixation/Permeabilization Kit Protocol

A Solutions and Reagents

NOTE: Precipitation may occur in some kit components. The presence of precipitate does not affect the performance of the reagent.

SUPPLIED REAGENTS

- **FoxP3/Transcription Factor Fixation/Permeabilization Diluent (1X)** (#58766)
- **FoxP3/Transcription Factor Fixation/Permeabilization Concentrate (4X)** (#44931): Dilute desired amount to a 1X working solution with FoxP3/Transcription Factor Fixation/Permeabilization Diluent (1X)
- **FoxP3/Transcription Factor Permeabilization Buffer (10X)** (#68751): Dilute desired amount to a 1X working solution with reverse osmosis deionized (RODI) or equivalent grade water. Use for all wash steps and antibody incubation following fixation.

ADDITIONAL REAGENTS (NOT SUPPLIED)

- Red blood cell lysis buffer
- Unconjugated or fluorochrome-conjugated primary antibodies
- Fluorochrome-conjugated secondary antibodies (if applicable)
- Cellular dyes (e.g., live-dead discrimination, DNA dyes, etc.), optional. Visit www.cellsignal.com/flowdyes for a full listing of flow cytometry validated cellular dyes.

B Fixation

NOTE: Fixable viability dyes such as Ghost Dye™ Violet 510 Viability Dye #59863 should be added prior to fixation, following the dye product recommended protocol. Proceed with fixation once excess dye has been removed.

NOTE: Add antibodies targeting CD markers or other extracellular epitopes prior to fixation. The antibodies will remain bound to the target of interest during the fixation process. A wash step prior to fixation may be performed but is not necessary.

1. Pellet cells by centrifugation and aspirate supernatant.
2. Resuspend cells in 1 mL FoxP3/Transcription Factor Fixation/Permeabilization 1X working solution, prepared as described above. Mix well to dissociate pellet and prevent cross-linking of individual cells.
3. Incubate for 1 hr at room temperature (20-25 °C). Protect from light.
4. Wash cells 2 times in excess 1X FoxP3/Transcription Factor Permeabilization Buffer.

C Immunostaining

1. Aliquot desired number of cells into tubes or wells.
2. Resuspend cells in 100 µl of diluted antibody, prepared in 1X FoxP3/Transcription Factor Permeabilization Buffer at the recommended dilutions. See individual antibody datasheets or product webpage for recommended dilution, or determine via titration.
3. Incubate for 1 hr at room temperature (20-25 °C). Protect from light.
4. Wash by centrifugation in excess 1X FoxP3/Transcription Factor Permeabilization Buffer. Discard supernatant. Repeat.
5. If using a fluorochrome-conjugated primary antibody, resuspend cells in 500 µl 1X FoxP3/Transcription Factor Permeabilization Buffer and analyze on flow cytometer; for unconjugated primary antibodies, proceed to next step.
6. Resuspend cells in fluorochrome-conjugated secondary antibody, diluted in 1X FoxP3/Transcription Factor Permeabilization Buffer at the recommended dilution.
7. Incubate for 30 min at room temperature (20-25 °C). Protect from light.
8. Wash by centrifugation in excess 1X FoxP3/Transcription Factor Permeabilization Buffer. Discard supernatant. Repeat.
9. Resuspend cells in 500 µl 1X FoxP3/Transcription Factor Permeabilization Buffer and analyze on flow cytometer.