Store at 4°C

Intracellular Flow Cytometry Kit (Triton™ X-100)

#51995

1 Kit (100 tests)

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

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

New 07/19

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

Applications	Species Cross-Reactivity
F	All

Products Included	Product #	Volume	Storage Temp
10X Wash Buffer, Phosphate Buffered Saline (PBS)	12528	125 ml	Room Temp.
4% Formaldehyde, Methanol-Free	47746	20 ml	Room Temp.
Cell Permeabilization Buffer (Triton™ X-100)	39487	20 ml	4°C
Flow Cytometry Antibody Dilution Buffer	13616	25 ml	4°C

Description: The Intracellular Flow Cytometry Kit (Triton™ X-100) provides the supporting reagents needed to preserve protein states and enable antibodies to bind intracellular targets, for flow cytometric analysis of cells in suspension. Detergent-based permeabilization provides access to the nucleus and other intracellular organelles, without affecting fluorophores of direct conjugates added prior to fixation. This kit contains sufficient reagents for 100 individual samples when following the included protocol.

IMPORTANT: Please refer to the antibody product page to determine if it is validated for use in Flow Cytometry (F) and for information regarding appropriate antibody dilution. Some primary antibodies may require methanol permeabilization, which will be noted on the datasheet. Methanol is not included in this kit.

Storage: All components in this kit are stable for at least 12 months when stored at the recommended temperature and left unused. Formaldehyde fixative should be used within one month after opening.

Upon receipt, #13616 and #39487 should be stored at 4°C. Remaining components should be stored at room temperature.

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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NOTE: Prior to using this kit, refer to the product webpage for antibodies of interest to determine whether they are validated for Flow Cytometry (F) assays using a Triton™ X-100 permeabilization protocol.

NOTE: When used in conjunction with fluorescent cellular dyes (including viability dyes, DNA dyes, etc.), please refer to the dye product page for recommended protocol. Visit www.cellsignal.com/flowdyes for a listing of flow cytometry validated cellular dyes.

A Solutions and Reagents

SUPPLIED REAGENTS

1. **10X Wash Buffer, Phosphate Buffered Saline (PBS)** (#12528): Dilute desired amount to a 1X working solution with reverse osmosis deionized (RODI) or equivalent grade water, mix. Store at 4°C and use within one month once diluted.
2. **4% Formaldehyde, methanol-free** (#47746): Store at room temperature. Use within one month once opened.
3. **Cell Permeabilization Buffer (Triton™ X-100)** (#39487): Store at 4°C.
4. **Flow Cytometry Antibody Dilution Buffer** (#13616): Store at 4 °C.

ADDITIONAL REAGENTS (NOT SUPPLIED)

1. Red blood cell lysis buffer
2. Unconjugated or fluorochrome-conjugated primary antibodies
3. Fluorochrome-conjugated secondary antibodies (if applicable)
4. Cellular dyes (e.g., live-dead discrimination, DNA dyes, etc.), optional

NOTE: Not all extracellular antibodies will bind correctly after fixation and/or detergent permeabilization, so a small-scale experiment should be performed if you are unsure. Antibodies targeting CD markers or other extracellular proteins may be added 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.

B Fixation

NOTE: Adherent cells or tissue should be dissociated and in single-cell suspension prior to fixation.

1. Pellet cells by centrifugation and remove supernatant.
2. Resuspend cells in approximately 100 µl 4% Formaldehyde (#47746) per 1 million cells. Mix well to dissociate pellet and prevent cross-linking of individual cells.
3. Fix for 15 min at room temperature (20-25 ºC).
4. Wash by centrifugation with excess 1X Wash Buffer. Discard supernatant containing formaldehyde in appropriate waste container. Repeat.
5. Proceed to Permeabilization step, or store overnight in 1X PBS at 4°C.

C Permeabilization

1. If necessary, pellet cells by centrifugation and aspirate supernatant.
2. Permeabilize cells by adding 100 µL Cell Permeabilization Buffer (Triton™ X-100) (#39487) per 1 million cells.
3. Permeabilize for a minimum of 10 minutes.
4. Proceed to Immunostaining step, or store cells overnight in 1X PBS at 4°C.

D Immunostaining

1. Aliquot desired number of cells into tubes or wells. (Generally, 5 x 10^5 to 1 x 10^6 cells per assay.)
2. Pellet cells by centrifugation and remove supernatant.
3. Resuspend cells in 100 µl of diluted antibody or antibody conjugates, prepared in Flow Cytometry Antibody Dilution Buffer (#13616) at the recommended dilutions. See individual antibody datasheets or product webpage for recommended dilution, or determine via titration.
4. Incubate for 1 hr at room temperature (20-25°C).
5. Wash by centrifugation in excess 1X Wash Buffer. Discard supernatant. Repeat.
6. If using a fluorochrome-conjugated primary antibody, resuspend cells in 200-500 µl 1X Wash Buffer and analyze on flow cytometer; for unconjugated primary antibodies, proceed to next step.
7. Resuspend cells in fluorochrome-conjugated secondary antibody, diluted in Flow Cytometry Antibody Dilution Buffer (#13616) at the recommended dilution.
8. Incubate for 30 min at room temperature (20-25°C).
9. Wash by centrifugation in excess 1X Wash Buffer. Discard supernatant. Repeat.
10. Resuspend cells in 200-500 µl 1X Wash Buffer and analyze on flow cytometer.