Store at 4°C

#13593

Intracellular Flow Cytometry Kit (Methanol)

1 Kit (100 tests)

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

Orders: 877-616-2355 (U.S.)
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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.
Methanol	13604	2 x 25 ml	Room Temp.
Flow Cytometry Antibody Dilution Buffer	13616	25 ml	4°C

Description: The Intracellular Flow Cytometry Kit provides the supporting reagents needed to preserve protein states and enable antibodies* to bind intracellular targets, for flow cytometric analysis of cells in suspension. This kit contains sufficient reagents for 100 individual samples when following the supplied 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 detergent permeabilization, which will be noted on the datasheet. Detergent 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 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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Intracellular Flow Cytometry Kit (Methanol) Protocol

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 methanol 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 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. **100% Methanol (#13604):** Keep at room temperature for long-term storage. Chill to -20°C before use.
4. **Flow Cytometry Antibody Dilution Buffer (#13616):** Store at 4°C.

Additional Reagents (Not Supplied):

1. Red blood cell lysis buffer (#46232)
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

B. Fixation

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

NOTE: Antibodies targeting CD markers or other extracellular proteins may be added prior to fixation if the epitope is disrupted by formaldehyde and/or methanol. The antibodies will remain bound to the target of interest during the fixation and permeabilization process. However, note that some fluorophores (including PE and APC) are damaged by methanol and thus should not be added prior to permeabilization. Conduct a small-scale experiment if you are unsure.

1. Pellet cells by centrifugation and remove supernatant.
2. Resuspend cells in approximately 100 µl 4% formaldehyde 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°C).
4. Wash by centrifugation with excess 1X PBS. Discard supernatant in appropriate waste container. Resuspend cells in 0.5-1 ml 1X PBS. Proceed to Permeabilization step.
   a. Alternatively, cells may be stored overnight at 4ºC in 1X PBS.

C. Permeabilization

1. Permeabilize cells by adding ice-cold 100% Methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol.
2. Permeabilize for a minimum of 10 min on ice.
3. Proceed with Immunostaining or store cells at -20°C in 90% Methanol.

D. Immunostaining

NOTE: Count cells using a hemocytometer or alternative method.

1. Aliquot desired number of cells into tubes or wells. (Generally, $5x10^5$ to $1x10^6$ cells per assay.)
2. Wash cells by centrifugation in excess 1X PBS to remove methanol. Discard supernatant in appropriate waste container. Repeat if necessary.
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 (20-25ºC).
5. Wash by centrifugation in excess 1X Wash Buffer. Discard supernatant. Repeat.
6. If using fluorochrome-conjugated primary antibodies, 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 (20-25ºC).
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.

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