Product Numbers: KF37200, KF37201, KF37202

IntraCyte™ Intracellular FACS Kit

IntraCyte kits are intended for the fixation, permeabilization, and detection of intracellular antigens by flow cytometry in single cell suspensions. IntraCyte Fixative Solution is a formaldehyde-based fixative containing a small amount of organic solvents which provide superior preservation of cellular morphology and antigenicity. IntraCyte Wash Solution permeabilizes and washes using a proprietary detergent blend which maximizes signal intensity and reduces background. The kit provides everything required except for cells, antibodies, PBS, staining tubes, centrifuge, and a flow cytometer. IntraCyte Ms-FITC and Rb-PE kits also provide pre-titered and optimized fluorochrome-conjugated secondary Abs. WARNING: fixative contains formaldehyde, a known carcinogen (consult MSDS for handling precautions).

Kit Components

- IntraCyte Fix Solution (Cat # K37203), 150 mls, ready to use
  - ***Store at 4-8°C***
- IntraCyte Wash Solution (Cat # K37205), 300 mls, ready to use
  - ***Store at 4-8°C***
- IntraCyte Block Reagent (Cat # K37206), 3.5 mls, 20X solution
  - ***Store at 4-8°C***

IntraCyte Ms-FITC also includes:
- pre-titered anti-Mouse FITC labeled 2nd antibody

IntraCyte Rb-PE also includes:
- pre-titered anti-Rabbit PE labeled 2nd antibody

Quality Control

Primary human dermal fibroblasts in culture are processed following the protocol below. Tests are conducted for preservation of cellular morphology by FSC vs. SSC, background IgG binding, and antigen detection by intracellular FACS.

Protocol Outline

- **Count cells and wash** >1e6 viable cells by centrifugation in 10-15 mls ice cold PBS
- **Fix in IntraCyte Fix** by re-suspending and incubate >30 minutes at Room Temp. or 4°C
- **Wash in PBS** by addition of 10-15 mls then centrifuge as before
- **Re-suspend in IntraCyte Wash** 10-15 mls, then centrifuge as before
- **Re-suspend in IntraCyte Wash** 1-2 mls, then add 50-100 uL 20X IntraCyte Block and mix
- **Stain** 0.1-0.2 mls/test by addition of primary Abs/controls for 30 minutes at Room Temp
- **Wash in IntraCyte Wash** by addition of 1.0 to 1.5 mls/test IntraCyte-Wash solution, then centrifuge as before
- **Re-suspend in IntraCyte Wash with 1X Block,** 0.1-0.2 mls/test
- **Stain** with secondary Ab, wash as before by addition of IntraCyte-Wash and centrifugation
- **Re-suspend** in PBS and proceed to FACS analysis

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Detailed Protocol

1. **Wash 1e6 to 5e6 viable cells in a single-cell suspension using 10-15 mls ice cold PBS by centrifugation at 200 to 300g for 5 minutes.** Protein must be removed prior to fixation. BSA or serum for example will interfere with the fixation reaction. A 15 ml conical centrifuge tube is convenient, since subsequent washing volumes of 10-15 mls will be used. Hank's Balanced Salt Solution can also be used.

2. **Fix cells by re-suspension in IntraCyte Fix Solution and incubate at Room Temp for 30 minutes, or overnight at approximately 4-8°C.** Fix 1e6 to 5e6 cells per 1.0 ml of fixative, scaling up volumes for larger cell numbers. Some cells and antigen/antibody combinations require longer fixation times at lower temperatures, while most antigens are efficiently preserved after 30 minutes at Room Temp. You should determine the optimal fixation times and temperatures for your cells and antigen/antibody combinations.

3. **Wash fixed cells by addition 10-15 mls of PBS and centrifugation at 300-400g for 10 minutes.** The formaldehyde and organic solvent contained in the fixative must be removed prior to permeabilization using IntraCyte Wash. All steps from this point on can be performed at Room Temp. After fixation cells are less dense and therefore require slightly more force to efficiently pellet.

4. **Re-suspend in 1-2 mls using *IntraCyte*-Wash.** Using a P1000 tip helps in re-suspension, and a cell count can be performed as well to determine yield and volume of additional IntraCyte Wash with added Block that can be used. It is generally most convenient to stain for FACS using 0.1-0.2 mls per test with 1e5 to 5e5 cells in each test in a 1.5ml microfuge tube. 96-well format can also be used, and we recommend using 50 uL with 1e5 to 5e5 cells per well.

5. **Add 50-100 uL 20X *IntraCyte*-Block and mix gently.** *IntraCyte*-Block is provided as a 20X stock and should be added after re-suspension of cells to a final of about 1x concentration. Cells can be stored for up to 4 months at this point at 4-8°C.

6. **Add appropriate concentration of primary antibody in 1-10 µL and mix gently.** Dilute primary antibody as needed prior to addition using *IntraCyte* Wash. Each primary antibody used should be carefully titered against your cells in your hands. It is generally convenient to stain each FACS test in a 1.5 ml microfuge tube.

7. **Incubate 30-60 minutes at Room Temp, then wash cells by addition of 1.0-1.5 mls of *IntraCyte*-Wash and centrifugation at 300g for 5 minutes** Use at least 1 ml per test for 1.5 ml tubes and 0.3 ml per test for 96 well plates. Only a single wash step is usually required, but sometimes washing twice can reduce background. When aspirating remember to leave a small volume of wash fluid above the cell pellet.

8. **Re-suspend in 0.1 ml per test of *IntraCyte*-Wash complete with added 20x *IntraCyte*-Block.**

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9. **Stain with pre-titered fluorochrome-conjugated secondary antibody at appropriate concentration.** For best results use *IntraCyte* Ms-FITC (Cat # KF37201) and *IntraCyte* Rb-PE Secondary antibody kits (Cat # K37202) at 10 μL per test. *******Other secondary Ab's must be evaluated for compatibility, and specificity, and titered to determine appropriate concentration for intracellular FACS.***

10. **Incubate 30-60 minutes at Room Temp, then wash cells by addition of 1.0-1.5 mls of *IntraCyte*-Wash and centrifugation at 300g for 5 minutes.**

11. **Re-suspend in 0.1 to 0.2 ml per test of FACS storage buffer.** Store in dark at approximately 4°C for up to 1 week prior to FACS analysis. FACS storage buffer can be made by diluting IntraCyte-Fix 1/50 in PBS.

12. **FACS setup tips:**

    a. Intracellular FACS generally gives higher levels of non-specific IgG binding; use an irrelevant IgG control as an "unstained" sample, reducing the voltage to bring this population into the first or second decade on a log scale, to correct for this effect.

    b. Spiking some cells from your irrelevant IgG controls into your positive control sample can aid in getting the appropriate voltage and compensation parameters in the flow cytometer.

    c. Both single-color controls for compensation and irrelevant IgG controls MUST BE RUN FOR EACH EXPERIMENT.

    d. Titer all primary and secondary Abs

Immunofluorescent Staining Protocol

IntraCyte reagents can also be used for immunofluorescence on either cells or tissue sections. Just follow the same steps as in the intracellular FACS protocol, changing volumes as necessary while maintaining ratios, times, and temperatures. For example, if you have cultured cells in chamber slides, wash once in PBS, fix cells in IntraCyte Fix Solution, wash out fixatives with PBS, permeabilize with IntraCyte Wash Solution, add IntraCyte 20X Block Reagent, stain with primary Ab, wash with IntraCyte Wash Solution, add IntraCyte 20X Block Reagent, stain with secondary fluorochrome-conjugated Ab, wash as before and mount for microscopy.

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