Virongy p24 Intracellular protein Staining Protocol

Virongy's Intracellular Protein Staining Kit provides a flexible and reliable tool for immunofluorescent staining of intracellular host and viral proteins, such as host phospho-proteins and viral capsid proteins. Cells are chemically fixed with a Fixation Buffer to stop proteolytic enzymes, prevent microbial contamination, and preserve cell morphology. **Permeabilization Buffer** dissolves fatty acids in the cell membrane to allow antibodies to access intracellular proteins. **Staining/Washing Buffer** improves immunofluorescent staining of cells by reducing non-specific binding and antigen capping. After staining, cells are ready for fluorescence-activated cell sorting and analysis. Virongy's intracellular staining kit is compatible with various combinations of antibodies and cytokines to accommodate your individual research needs.

Key Features:

- Compatible with a variety of cell types. Single cells can be analyzed quantitatively for multiple parameters at the same time.
- Simple protocol, yielding reliable results. In just three simple steps – fixation, permeabilization, and staining – cells are ready for fluorescence-activated cell sorting and analysis.

Kit includes:

- Virongy **Fixation Buffer:** 250 ml
- Virongy **Permeabilization Buffer**: 250 ml
- Virongy **Staining/Washing Buffer:** 250 ml

Procedure:

1. Aliquot approximately 1 x 10^6 cells in 1 ml of cold PBS into a 5 ml staining tube.
2. Fix cells by adding an equal volume (1 ml) of **Fixation Buffer** directly into the cell, mix well, and incubate for 10 min at room temperature.
3. Pellet cells by centrifugation for 2,000 rpm (or 600 x g) for 5 minutes at room temperature. Discard the supernatant.
4. Permeabilize cells by resuspending in 1 ml **ice-cold Permeabilization Buffer** with vigorous vortexing, incubate at 4°C for at least 30 min (**Note:** A*t this step, cells can be stored in* **Permeabilization Buffer** *at -20℃ for up to 5 weeks*).
5. Wash cells twice with 2 ml **Staining/Washing Buffer**.
6. Discard the supernatant. Leave about 100 - 150 µl of **Staining/Washing Buffer** in the tube.
7. Add 5 µl of FITC labelled p24 IgG2a,k antibody. (Dilution is made in **Staining/Washing Buffer**).

VBI-HBK-007 Rev 001
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Dilution A: Undiluted Ab
Dilution B: 1:10 dilution

8. Incubate at room temperature for 60 minutes.
9. Wash cells 3 times with 2 ml **Staining/Washing Buffer**. Pellet cells by centrifugation for 2,000 rpm (or 600 x g) for 5 minutes at room temperature.
10. Resuspend cells in 500 µl **Staining/Washing Buffer** and analyze by FACS.

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