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Minute™ Cell Suspension Isolation Kit from Fresh/Frozen Tissues

Cat. No. CS-031

Description

Obtaining quality cell suspension from fresh and/or frozen tissues is an important first step for subsequent analysis such as flow cytometry analysis (FACS) and nucleic acid purification. Cell suspensions can be isolated from tissues by one or a combination of the following three mechanisms: chemical tissue dissociation, enzymatic digestion and physical separation. Many methods are tedious and time consuming. Most importantly, cells in the tissue especially frozen tissue are usually damaged and show low integrity and viability. It is very difficult to obtain high viability cell suspension from frozen tissues with high connective tissue contents such as liver, kidney and brain tissues. We have developed a simple, rapid, efficient, and instrument-free method for isolating cell suspension from fresh or frozen tissues using a combination of chemical tissue dissociation and physical separation mechanisms. The protocol can be finished in about 20 min with higher cell integrity and viability.

Applications

The cell suspension obtained with the kit can be used for FACS analysis and other applications.

Shipping and storage: The kit is shipped at ambient temperature and stored at 4-8^{o}C

Kit components

- Tissue dissociation buffer 15 ml
- 1.5 ml microfuge tube X 50
- Pestle for 1.5 ml tube X 2

Materials required but not included in the kit

Table top microcentrifuge. FACS buffer (5% BSA, 0.01% sodium azide in 1 X PBS). 40-100 µm cell strainer

Protocol (Chill tissue dissociation buffer and FACS buffer on ice prior to use)

1. Place 10-30 mg tissue in a 1.5 ml tube provided. Add 200 µl cold tissue dissociation buffer to cover the tissue, incubate on ice for 5 -10 min. Gently grind the tissue for 100-200 times with the pestle provided using back and forth twisting force (this step takes about 2-3 min). The pestle is reusable, for cleaning, rinse it with tape water and dry with paper towel. It is not

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recommended to use 1.5 ml tubes from another source because they may not fit the pestle properly.

2. After grinding, centrifuge the tube at 500 X g for 3 min. Remove and discard the supernatant. Grind the wet pellet with the pestle for about 100-150 times with twisting force. Leave the pestle in the tube and add 1 ml FACS buffer to the tube. Grind the sample a few more times to resuspend. Clean and dry the pestle as described above for future use.

3. Resuspend the cells by pipetting up and down using a 1 ml pipette tip for about 10 to 20 times. Pass the cell suspension through a 40-100 µm cell strainer depending upon the final cell size desired. Harvest cells by low speed centrifugation (500 X g for 3 min) and resuspend the cell pellet in proper amount of FACS buffer. Don't resuspend the cells in PBS because the cells are very fragile. The viability of the cells will be significantly reduced if they are not resuspended in FACS buffer or other serum containing buffers such as tissue culture medium with 10-20% bovine fetal serum.