/ CONTEXT

Flow cytometry is often used to study cells for immunity or cancer research. Obtaining viable single cell suspension is the first step before performing flow cytometry. Although, there are many protocols and methods reporting tissue dissociation, most common techniques are enzymatic and mechanical disruption. The main advantage of mechanical disruption is the ability to contain metabolically active cells, in addition to being faster in comparison with enzymatic approach.

In this study, a high viability of living cells in a single cell suspension were obtained using bead beating technology operated by the Minilys® within 5 seconds.

/ MATERIALS

- Minilys® cell homogenizer by Bertin Instruments
- Precellys® lysing kit: CKMIX50 7 ml KT03961-1-306.7
- Sample: 300 mg of fresh chicken liver
- Buffer: 1 ml of sterile PBS
- Cellometer ViaStain™ AOPI Staining Solution in PBS
- InCell® Cell Imaging system by Bertin Instruments
- InCell® 40X Obj. -#LCACHN-PH40x

/ PROTOCOL

Minilys® parameters:
The 300 mg liver (+ 1ml of PBS) is homogenized in a 7 ml tube prefilled with ceramic beads.
The Minilys® is set up at lower speed (3000 rpm) for 5 sec.

Viability control with AOPI staining:
After homogenization, 20 μl of cell suspension (with no extra sample preparation step) is gently mixed with 20 μl of AOPI staining solution in a new 1.5 ml tube.

Visualization with InCell®:
A drop of the stained cell suspension is observed upon a slide cover with a coverslip.

/ CONCLUSION

The Minilys® combined with the dedicated Lysing Kit efficiently dissociated tissue. In only 5 seconds, this process enables to obtain a metabolically active single cell suspension.

Single-use consumables avoid any cross-contamination between samples.
The InCell® contributes to control the quality of the cell suspension, providing publication quality images of cells with no extra sample preparation step after staining with AOPI.