Minute™ Single Cell Isolation Kit

(Non-Sterile)

Catalog number: SC-012

Description

Minute™ single cell isolation kit comprises optimized tissue disaggregation buffers and specially designed filter cartridges with 2.0 ml collection tubes. The kit is designed to isolate single cells from fresh animal tissues rapidly, and it can also isolate nuclei from tissues briefly fixed with formaldehyde for chromosome immunoprecipitation (ChIP). The tissue disaggregation buffers are formulated to disaggregate animal tissues gently. The buffers don't contain any proteinases and EDTA that may adversely affect cell surface markers. Due to filter cartridges with pre-defined pore size and a specially formulated buffer system, single-cell suspension can be obtained from fresh tissues in less than 10 min. This kit is specifically designed for lymphoid tissues such as the spleen, thymus, and lymph node. For other tissues, the performance is tissue type-dependent. For cell suspension isolation from frozen/fresh tissues, please refer to Cat# CS-031.

Application

Single cells isolated with this kit can be used as starting materials for FACS and chromosome immunoprecipitation (ChIP) analysis. The single-cell suspension can also be used as a starting material for isolation/purification of DNA, RNA, proteins, and other cellular components.

Kit components

1. 25 ml buffer A (for non-fixed tissues)
2. 25 ml buffer B (for formaldehyde-fixed tissues)
3. 50 protein extraction filter cartridges
4. 50 collection tubes with cap
5. Plastic rod (2)

Shipping: This kit is shipped at ambient temperature
Storage: Store at 4°C upon arrival

Additional Materials Required

Table-Top Microcentrifuge
1 X PBS or FACS buffer (1 X PBS with 5% FBS or BSA)
1.25 M glycine (for isolating nuclei from fixed tissues)
37% formaldehyde (for isolating nuclei from fixed tissues)

Choice of buffers:

Buffer A: for fresh tissues;
Buffer B: for formaldehyde-fixed tissues (fixation time should be less than 20 min).

Protocol for isolating single cells from fresh tissue (10-40 mg tissue/sample)
(Number of preps: 50)

1. **Prior to use, add fetal bovine serum (FBS, not provided) to buffer A (100 µl FBS to 1 ml buffer)**. Pre-chill buffer A and a filter cartridge in the collection tube on ice.
2. Place tissue in the filter. Add 100 µl cold buffer A to the filter, grind the tissue with a plastic rod for 50-60 times with twisting force (Note: The plastic rod is reusable. For cleaning, rinse it thoroughly with distilled water and dry it with a paper towel).
3. Add an additional 400 µl buffer A to the filter, cap the filter, invert a few times, and centrifuge in a microcentrifuge at 1200 X g for 2-3 min.
4. Resuspend the pellet by vortexing and centrifuge at 400 X g for 5 min. Discard the supernatant and resuspend the pellet (isolated single cells for fresh tissue) in a cold tissue culture medium that contains 10-20% BSA, FACS buffer, or other buffers of your choice.

Protocol for in-filter tissue fixation with formaldehyde for isolating nuclei (for ChIP)
(Number of preps: 25)

1. Pre-chill buffer B and a filter cartridge in collection tube on ice.
2. Weight tissues (10-40 mg, fresh or frozen) and chop tissue into small pieces (about 1-3 mm^3) using a sharp blade
3. Transfer tissue into a filter cartridge (filter A) in a collection tube and add 0.5 ml cold PBS and 14 µl formaldehyde (37%) to the filter. Cap the filter and inverting a few times and incubate at RT for 15 min. Invert the tube every 5 min.
4. Add 50 µl of 1.25 M glycine to the filter, cap the filter, invert the tube a few times, and incubate at RT for 5 min. Centrifuge at 2000 X g for 10 seconds, and wash the tissue once with 0.5 ml PBS. Discard the flow-through.
5. Add 100 µl of cold buffer B to the filter cartridge, grind the tissue with the plastic rod for 50-60 times with twisting force (Note: The plastic rod is reusable. For cleaning, rinse it thoroughly with distilled water and dry it with a paper towel). Add additional 400 µl of buffer B to the filter cartridge (in collection tube) and place it on ice for 2-3 min to allow larger un-disaggregated tissue debris to settle.
6. Carefully transfer 400 µl supernatant from filter A to a new filter (designated as filter B) with a collection tube. Add 300 µl buffer B to filter A. Cap the filters A and B, invert a few times and centrifuge in a microcentrifuge at 2000 X g for 4-5 min (Optional: transfer the supernatant from the collection tube and resuspend residue tissue homogenate on the filter and centrifuge at 2000 X g for 4-5 min to further increase the yield).
7. Resuspend the pellet by vortexing and centrifuge at 1200 for 4-5 min. Discard the supernatant and resuspend the pellet (isolated nuclei) in the buffer of your choice. The isolated nuclei can be used for ChIP.

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