Minute™ Single Cell Isolation Kit

(Non-Sterile)
Catalog number: SC-012

Description

Invent Biotechnologies Minute™ single cell isolation kit is composed of optimized tissue disaggregation buffers and specially designed filter cartridges with 2.0 ml collection tubes. The kit is designed to rapidly isolate single cells from fresh animal tissues. It can also be used for isolating nuclei from tissues briefly fixed with formaldehyde for chromosome immunoprecipitation (ChIP). The tissue disaggregation buffers are formulated to gently disaggregate animal tissues. The buffers don't contain any proteinases and EDTA that may have adverse effects on cell surface markers. Due to the use of filter cartridges with pre-defined pore size and 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 spleen, thymus, and lymph node. For other tissues the performance is tissue type-dependent. For isolation of cell suspension isolation from frozen/fresh tissues please refer to Cat. No. 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)

Choice of buffers:

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

Protocol (1-40 mg tissue/filter cartridge)

1. **Prior to use add fetal bovine serum (FBS, not provided) to the buffer (100 µl FBS to1 ml buffer)**. Pre-chill buffer (s) and a filter cartridge in collection tube on ice.
2. Place tissue in the filter. Add 100 µl cold buffer 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 paper towel).
3. Add 400 µl buffer (the same buffer as used in step 2) to the filter, cap the filter and 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 supernatant and resuspend the pellet (isolated single cells for fresh tissue)/(nuclei for fixed tissue) in cold tissue culture medium that contains 10-20% BSA ,FACS buffer or other buffers of your choice.

Optional Protocol for in filter tissue fixation with formaldehyde
(Reagents required but not included: 37% formaldehyde, 1.25 M glycine)

1. Pre-chill buffer (s) and a filter cartridge in collection tube on ice.
2. Weight frozen or fresh tissues (10-40 mg)
3. Chop tissue into small pieces using 2 razor blades (between 1-3 mm3).
4. 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. Inverting the tube every 5 min.
5. Add 50 µl 1.25 M glycine to the filter, cap the filter and invert the tube a few times and incubate at RT for 5 min. Centrifuge at 2000 X g for 10 seconds, wash the tissue once with 0.5 ml PBS. Discard the flow through.
6. Add 100 µl cold buffer B 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 paper towel). Add 400 µl buffer B to the filter and place the tube on ice for 2-3 min to allow larger un-disaggregated tissue debris to settle.
7. Carefully transfer 400 µl supernatant from filter A to a new filter (this is designated filter B) with 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 yield).
8. Resuspend the pellet by vortexing and centrifuge at 1200 for 4-5 min. Discard supernatant and resuspend the pellet (isolated nuclei) in the buffer of your choice.

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