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/nuclei from fresh/frozen/formaldehyde fixed animal tissues. The tissue disaggregation buffers are formulated to gently disaggregate animal tissues. The buffers don't contain any proteinases that may have adverse effects on cell surface marker detection. Due to the use of filter cartridges with pre-defined pore size and thickness, single cell suspension can be isolated from fresh or formaldehyde fixed tissues in less than 8 min with high yield.

Application

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

Buffer Formulation: Proprietary

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 (4)

Storage: Store the kit at $4^{O}C$

Additional Materials Required

Table-Top Microcentrifuge
1 X PBS or FACS buffer (1 X PBS with 5% FBS or BSA)

Single Cell Isolation Protocols

Following procedures are for isolation of single cell suspension from 2-60 mg fresh/frozen/formaldehyde fixed animal tissues. **Note: For single cell isolation from non-fixed fresh/frozen tissues use buffer A. For single cell isolation from formaldehyde-fixed tissues use buffer B for tissue disaggregation.**

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Protocol A (for tissue <30 mg)

1. Pre-chill buffer (s) and a filter cartridge in collection tube on ice.
2. Place tissue (2-29 mg) 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). Optional: add 50 µl serum-containing tissue culture medium to 100 µl buffer may increase cell viability).
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 5,000 rpm for 2-3 min .
4. Remove the supernatant and resuspend the pellet (isolated single cells) in cold PBS, FACS buffer or any buffers suitable for downstream applications.

Protocol B (for tissue between 30-60 mg)

1. Pre-chill buffer (s) and a filter cartridge in collection tube on ice.
2. Place tissue (30-60 mg) in the filter (this is designated filter A). 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). Add 400 µl buffer (the same buffer as used in step 2) to the filter and place the tube on ice for 2-3 min to allow larger un-disaggregated tissue debris to settle.
3. Carefully transfer 400 µl supernatant from filter A to a new filter (this is designated filter B) with collection tube. Add 300 µl buffer to filter A. Cap the filters A and B, invert a few times and centrifuge in a microcentrifuge at 5,000 rpm for 2-3 min.
4. Remove the supernatants from both collection tubes and resuspend the pellets (isolated single cells) in cold PBS, FACS buffer or any buffers suitable for downstream applications.

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 (30-60 mg)
3. Chop tissue into small pieces using 2 razor blades (between 1-3 mm3).
4. Transfer tissue into a filter cartridge 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 5,000 rpm for 10 seconds, wash the tissue once with 0.5 ml PBS. Discard the flow through. Single cell suspension can be isolated from fixed tissue starting from protocol B step 2.

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