

# Minute™ Single Cell Isolation Kit

(Non-Sterile)

Catalog number: SC-012

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## 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 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.

**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°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.**

**Protocol A (for tissue <30 mg)**

1. **Prior to use add fetal bovine serum (FBS, not provided) to the buffer (100 µl FBS to 1 ml buffer).** 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).
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 4-5 min.
4. Remove the supernatant and resuspend the pellet (isolated single cells) in 1 ml cold tissue culture medium that contains 10-20% BSA or 1 ml FACS buffer.
5. Centrifuge at 3000rpm for 3 min, remove supernatant and resuspend the pellet in a buffer of your choice for downstream application

**Protocol B (for tissue between 30-60 mg)**

1. **Prior to use add fetal bovine serum (FBS, not provided) to the buffer (100 µl FBS to 1 ml buffer).** 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 4-5 min.
4. Remove the supernatants from both collection tubes and resuspend the pellets (isolated single cells) in 1 ml cold tissue culture medium that contains 10-20% FBS or 1 ml FACS buffer.
5. Centrifuge at 3000rpm for 3 min, remove supernatant and resuspend the pellet in a buffer of your choice for downstream application. If excessive cell debris is a concern, standard Ficoll Paque Media can be used to remove cell debris.

**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 mm<sup>3</sup>).
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.