10-min. Tissue (Tumor) Dissociation / Single Cell Isolation Kit (Non-Sterile)

Cat. #: P712-4 (4 rxn); P712-25 (25 rxn); P712-50 (50 rxn)

Storage: 4°C **Shelf Life:** 12 months

Product Description

This Tissue (Tumor) Dissociation / Single Cell Isolation Kit is designed to isolate single cells from 10-60 mg fresh/formaldehyde-fixed animal tissues (including tumor tissues), in less than 10 minutes. The tissue dissociation / disaggregation buffers are formulated to gently disaggregate animal tissues. The buffers do not contain any proteinases that may have adverse effects on cell surface marker detection.

The isolated single cells can be used for FACS, chromosome immunoprecipitation (ChIP), DNA/RNA/protein extraction or other cellular component isolation. This product is for research use only.

Product Components

Components	Amount			Storage
	Cat. #: **P712-4**	Cat. #: **P712-25**	Cat. #: **P712-50**	
Buffer A (for non-fixed tissue)	2 ml	12.5 ml	25 ml	4°C
Buffer B (for formaldehyde fixed tissue)	2 ml	12.5 ml	25 ml	4°C
Filter cartridges	4	25	50	Room temperature
Collection tubes with cap	4	25	50	Room temperature
Plastic rod	1	2	4	Room temperature

Additional Materials Required

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

Protocol:

Following procedures are for isolation of single cell suspension from 10-60 mg animal tissues.
For non-fixed, fresh tissues use buffer A. For formaldehyde-fixed tissues use buffer B. Fixation time should not more than 20 minutes.

Protocol A: for 10-29 mg tissue

1. **Prior to use, add fetal bovine serum (FBS, not provided) to the buffers (100 µl FBS to 1 ml buffer).** Pre-chill the buffer(s) and filter cartridge in collection tube on ice.
2. Place tissue (10-29 mg) in the filter. Add **100 µl cold buffer** to the filter, grind the tissue with a plastic rod for **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 at **4,000 rpm for 3-4 minutes**.

4. Now the single cells and the buffer are filtered into the collection tube. Do not aspirate. Discard the filter and resuspend the pellet by vortex. Then centrifuge at **2,000 rpm for 5 minutes**.

5. Aspirate and discard supernatant. Resuspend the pellet (isolated single cells) in cold tissue culture medium that contains 10-20% BSA or FACS buffer. The isolated single cells are ready for downstream assay.

Protocol B: for 30-60 mg tissue

1. **Prior to use, add fetal bovine serum (FBS, not provided) to the buffers (100 µl FBS to1 ml buffer).** Pre-chill the buffer(s) and filter cartridge in collection tube on ice.

2. Place tissue (30-60 mg) in the filter (we call it filter A). Add **100 µl cold buffer** to the filter, grind the tissue with a plastic rod for 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 and sit the tube on ice for 2-3 minutes to allow large un-disaggregated tissue debris to settle.

4. **Carefully transfer 400 µl supernatant** from filter A to a new filter (we call it filter B) in collection tube. Add another **300 µl buffer to filter A**. Cap both filters A and filter B, invert a few times and centrifuge them at **4,000 rpm for 3-4 minutes**.

5. Now the single cells and the buffer are filtered into the collection tubes. Do not aspirate. Discard the filters, resuspend the pellet by vortex and combine the single cell suspension from the two collection tubes. Then centrifuge at **2,000 rpm for 5 minutes**.
6. Aspirate and discard supernatant. Resuspend the pellet (isolated single cells) in cold tissue culture medium that contains 10-20% BSA or FACS buffer. The isolated single cells are ready for downstream assay.

Note: If excessive cell debris is a concern, standard Ficoll Paqu Media can be used to remove cell debris.

Protocol C: for in filter tissue fixation, 30-60 mg (Reagents required but not included: 37% formaldehyde, 1.25 M glycine)

1. Pre-chill buffer(s) and filter cartridge in collection tube on ice.
2. Weigh frozen or fresh tissues (30-60 mg)
3. Chop tissue into small pieces (between 1 to 3 mm^3) using 2 razor blades.
4. Transfer tissue into a provided filter cartridge (we call it filter A) in collection tube, add **0.5 ml cold PBS** and **14 µl formaldehyde (37%)** to the filter (final formaldehyde concentration is 1%). Cap the filter, invert a few times and incubate at room temperature (15-25°C) for 15 minutes. Invert the tube a few times every 5 minutes. The formaldehyde can be replaced by paraformaldehyde (PFA). The final PFA concentration should be 2%, in 0.5 ml PBS solution.
5. Add 50 µl 1.25 M glycine to the filter, cap the filter, invert the tube a few times and incubate at room temperature for 5 minutes. Centrifuge at 5,000 rpm for 10 seconds and discard the flow through. Add 0.5 ml

PBS to the filter to wash the tissue once. Centrifuge at 5,000 rpm for 10 seconds and discard the flow through. The tissue is fixed now and ready for single cell isolation as described in the following.

6. Add **100 µl cold Buffer B** to the filter, grind the tissue with a plastic rod for 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.)

7. Add **400 µl Buffer B** to the filter and sit the tube on ice for 2-3 minutes to allow large un-disaggregated tissue debris to settle.

8. **Carefully transfer 400 µl supernatant** from this filter (filter A) to a new filter (we call it filter B) in collection tube. Add another **300 µl Buffer B to filter A**. Cap both filters A and filter B, invert a few times and centrifuge them at **5,000 rpm for 3-4 minutes**.

   Optional: transfer the supernatant in the collection tubes back to the filters, resuspend the residue tissue homogenate in the filter by pipetting up and down, and then centrifuge at 5,000 rpm for 4-5 minutes to collect the flow through. This can further increase the yield.

9. Now the single cells and the buffer are filtered into the collection tubes. Do not aspirate. Discard the filters, resuspend the pellet by vortex and combine the single cell suspension from the two collection tubes. Then centrifuge at **2,000 rpm for 5 minutes**.

10. Aspirate and discard supernatant. Resuspend the pellet (isolated single cells) in the buffer of your choice, or in cold FACS buffer. The isolated single cells are ready for downstream assay.

    Remarks: This protocol is developed and validated by 101Bio's OEM partner. Spin column based protein extraction and cell. fractionation technologies were developed by 101Bio's OEM partner.

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P504	Nuclear protein kit	cells / tissues → nuclear & cytosol	native	6
P505	Detergent-free kit	cells → total protein	denatured / native	5
P506	Detergent-free kit	Tissues → total protein	denatured / native	5
P507	Mitochondria kit	cells / tissues → mitochondria	native/detergent-free	25
P508	Plant total protein	plant tissues → total protein	denatured / native	5
P510	Plant detergent-free	plant tissues → total protein	native	6
P511	Plant chloroplast kit	plant tissues → intact chloroplast		5
P512	Bacteria total protein	bacteria → total protein	denatured	2
P513	Nuclear envelope kit	cells → nuclear envelope	native	40
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P515	Thick cell wall microbes pro.	microbes → total protein	denatured / native	10
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