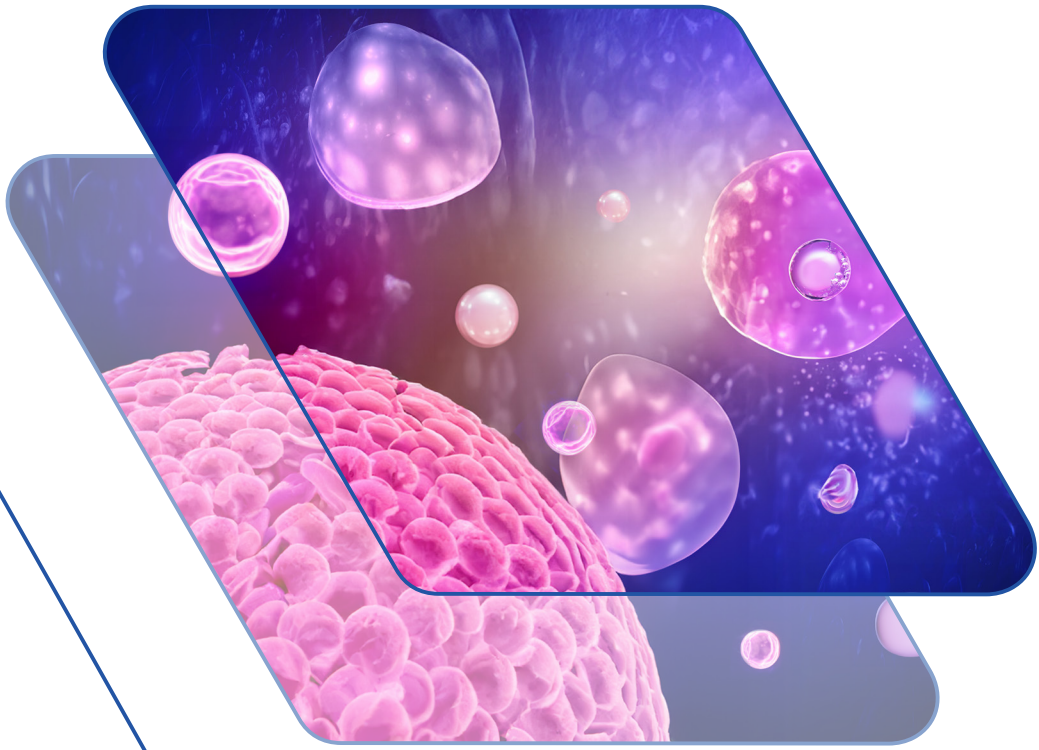
Precellys Multi Tissue Dissociation Kit (mouse)



USER GUIDE

Precellys Multi Tissue Dissociation Kit (mouse)

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Expiry date: stated on the package

Designation	Item #	Contence	Quantity
Precellys Dissociation Tubes	D30717	Tubes	20
Precellys Multi-Tissue Dissociation Kit	D05717	Enzyme MT-7	4x square bottles
		Enzyme MT-8	2x 2,5 mL Tubes

The Precellys Multi-Tissue Dissociation Kit contains enough reagents for 20 reactions.

Precellys Dissociation Tubes are not included and have to be purchased separately.

1. PRECAUTION FOR USE

Temperature

- Precellys Dissociation tubes must be stored at room temperature.
- Before opening the Precellys Multi-Tissue Dissociation Kit must be stored -20°C
- Enzyme MT7/Enzyme MT8 must be aliquots of appropriate volume to avoid thaw-cycles and store the aliquots at -20°C during maximum 6 months.

2. MATERIALS AND EQUIPMENT REQUIRED

In addition to standard laboratory equipment, the following materials may be required:

- Precision micropipettes (20 to 1000 µL)
- Incubator
- Precellys Evolution (P002511-PEVT0-A.0)
- 15 mL tube holder on the Precellys Evolution (P000810-PEVT0-A.0)
- DMEM/F-12, HEPES
- Fetal Bovine Serum (FBS)
- Phosphate-Buffered Saline (PBS) pH 7.4
- Red Blood Cell (RBC) Lysis buffer 1X
- PEB buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% Bovine Serum Albumin (BSA), and 2 mM EDTA.
- Debris Removal Solution (optional).

In function of the type of the tissue, the material and equipment required is not the same. For more information go to the specifical tissue protocol.

In this protocol, some steps will ask to run the following program on the Precellys Evolution instrument: 4500 rpm x 3 seconds.
Please note that if you don't have the latest software version you will have to manually stop the run at 3 seconds, as the minimum run in this version is 5 seconds.
Please refer to your local distributor to upgrade the software.

3. REAGENT PREPARATION

The dissociation enzymes (MT7/MT8) are ready to use solution.

4. DISSOCIATION OF MOUSE LIVER

1. Prepare enzyme mix for dissociation by pipetting the following reagents into a Precellys Dissociation Tube: 4875 µL of enzyme MT-7 and 125 µl of enzyme MT-8.
2. Transfer liver sample into the Precellys Dissociation Tube containing the enzyme mix. Close tightly.
3. Place the Precellys Dissociation Tube in the appropriate 15 mL tube holder on the Precellys Evolution.
4. Run the following program: 4500 rpm x 3 seconds.
5. Remove tube from Precellys Evolution and incubate sample for 30 minutes at 37 °C.
6. After incubation, place back the sample in the Precellys Evolution. Run the following program: 4500 rpm x 3 seconds.
7. Recover Precellys Dissociation Tube.
8. Resuspend sample and filter through a 100 µm cell strainer placed over a 50 mL conical tube.
9. Wash the filter with 5 mL of DMEM/F-12, HEPES.
10. Discard the filter and centrifuge cell suspension at 300xg for 10 minutes. Aspirate supernatant completely.
11. Resuspend cells with an appropriate buffer of the required volume for downstream application.

5. DISSOCIATION OF MOUSE LUNG

1. Prepare enzyme mix for dissociation by pipetting the following reagents into a Precellys Dissociation Tube: 4875 µL of enzyme MT-7 and 125 µl of enzyme MT-8.
2. Transfer lung sample into the Precellys Dissociation Tube containing the enzyme mix. Close tightly.
3. Place the Precellys Dissociation Tube in the appropriate 15 mL tube holder on the Precellys Evolution.
4. Run the following program: 4500 rpm x 3 seconds.
5. Remove tube from Precellys Evolution and incubate sample for 30 minutes at 37 °C.
6. After incubation, place back the sample in the Precellys Evolution. Run the following program: 4500 rpm x 3 seconds.
7. Recover Precellys Dissociation Tube.
8. Resuspend sample with 7.5 mL of DMEM/F-12, HEPES + 20% FBS.
9. Filter through a 70 µm cell strainer placed over a 50 mL conical tube.
10. Wash the filter with 3 mL of DMEM/F-12, HEPES + 20% FBS.
11. Discard the filter and centrifuge cell suspension at 600xg for 5 minutes. Aspirate supernatant completely.
12. Resuspend cells with an appropriate buffer to the required volume for downstream applications

6. DISSOCIATION OF MOUSE HEART

1. Prepare enzyme mix for dissociation by pipetting the following reagents into a Precellys Dissociation 4875 µL of enzyme MT-7 and 125 µl of enzyme MT-8.
2. Transfer heart sample into the Precellys Dissociation Tube containing the enzyme mix. Close tightly.
3. Place the Precellys Dissociation Tube in the appropriate 15 mL tube holder on the Precellys Evolution.
4. Run the following program: 4500 rpm x 3 seconds.
5. Remove tube from Precellys Evolution and incubate sample for 30 minutes at 37 °C.
6. After incubation, place back the sample in the Precellys Evolution. Run the following program: 4500 rpm x 5 seconds.
7. Recover Precellys Dissociation Tube.
8. Resuspend sample with 7.5 mL of DMEM/F-12, HEPES + 20% FBS.
9. Filter through a 70 µm cell strainer placed over a 50 mL conical tube.
10. Wash the filter with 3 mL of DMEM/F-12, HEPES + 20% FBS.
11. Discard the filter and centrifuge cell suspension at 600xg for 5 minutes. Aspirate supernatant completely.
12. Proceed with Debris removal step (optional - not included).
13. Proceed with Red blood cell lysis (section 6.2.3)

► **Red blood cell lysis**

1. Resuspend cell pellet in 1 mL of PEB buffer and add 10 mL of 1X Red Blood Cell Lysis buffer.
2. Incubate for 2 minutes at room temperature.
3. Centrifuge at 600xg for 5 minutes. Aspirate supernatant completely.
4. Resuspend cell pellet in 10 mL of PBS.
5. Centrifuge at 600xg for 5 minutes. Aspirate the supernatant completely.
6. Resuspend cells with an appropriate buffer to the required volume for downstream applications.

7. DISSOCIATION OF MOUSE SPLEEN/THYMUS

1. Prepare enzyme mix for dissociation by pipetting the following reagents into a Precellys Dissociation Tube: 4875 µL of enzyme MT-7 and 125 µl of enzyme MT-8.
2. Transfer spleen sample into the Precellys Dissociation Tube containing the enzyme mix. Close tightly.
3. Recover the dissociation tubes from Precellys Evolution. Incubate sample for 15 minutes at 37 °C.
4. After incubation, place back the sample in the Precellys Evolution. Run the following program: 4500 rpm x 2 cycles of 5 seconds, with a 10 second pause between cycles.
5. Recover the Precellys Dissociation Tube.
6. Resuspend sample with 7.5 mL of DMEM/F-12, HEPES and filter through a 30 µm cell strainer placed over a 15 mL conical tube.
7. Wash the filter with 2.5 mL of DMEM/F-12, HEPES.
8. Discard the filter and centrifuge cell suspension at 300xg for 10 minutes. Aspirate supernatant completely.
9. Resuspend cells with an appropriate buffer to the required volume for downstream applications.

NOTES

NOTE

Do not hesitate to contact our after-sales services for further information at

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