Miltenyi Biotec

Debris Removal Solution

Order no. 130-109-398

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1. Description

This product is for research use only.

Components	2× 45 mL Debris Removal Solution
Capacity	For 50 applications with 1 g of tissue per application.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Cell debris often occurs after tissue dissociation and impairs downstream applications.
The Debris Removal Solution has been developed for efficient removal of cell debris from all kind of tissue after dissociation. It has been particularly tested for debris removal from dissociated adult rat and mouse heart tissue and mouse tumor tissue.
In case of adult mouse or rat heart tissue, the Neonatal Heart Dissociation Kit, mouse and rat has been used for preparation of single-cell suspensions. For dissociation of mouse tumor tissue, the Tumor Dissociation Kit, mouse has been used.
The debris-free single-cell suspensions can be analyzed *in vitro* for phenotype distributions, and other functional, genetic, or proteomic studies. Furthermore, cells can be subsequently cultured or used for cell separation with MACS® Technology.

1.2 Applications

- Debris removal from dissociated tissue to improve downstream applications, such as cell separation, cell culture, cellular or molecular analysis.

1.3 Reagent and instrument requirements

- Dulbecco's phosphate-buffered saline (D-PBS)
- 15 mL reagent tubes
- (Optional) gentleMACS™ Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427)
- (Optional) gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) Tumor Dissociation Kit, mouse (# 130-096-730)
- (Optional) Neonatal Heart Dissociation Kit, mouse and rat (# 130-098-373)
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)

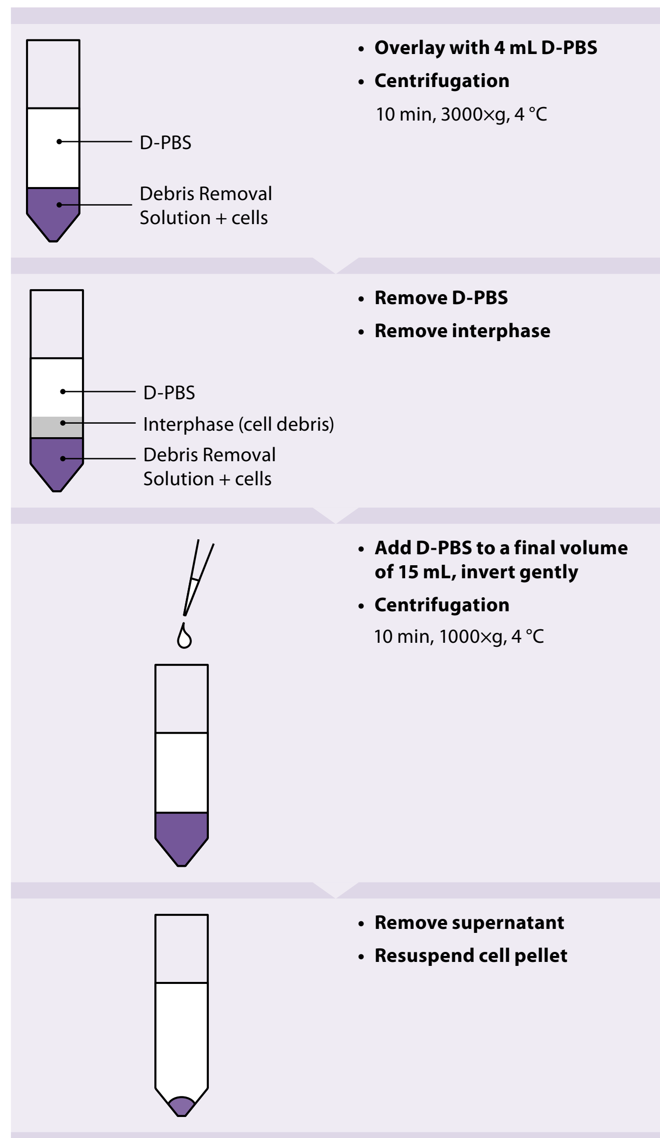

Figure 1: Debris removal protocol overview, steps 6–13

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2. Protocol

▲ Volumes given below are for debris removal from a cell suspension resulting from up to 1 g of starting material. When working with more than 1 g starting material, split the single-cell suspension in several reagent tubes. When working with less than 1 g, use the same volumes as indicated. When working with less than 0.5 g of tissue scale down all volumes according to the table below.

▲ For subsequent cell separation and cultivation it is recommended to dissociate at least 0.5 g of tissue.

▲ Always use pre-cooled buffers and solutions (4 °C).

	D-PBS	Debris Removal Solution	Overlay (D-PBS)
0.5–1 g tissue	6200 µL	1800 µL	4 mL
<0.5 g tissue	3100 µL	900µL	4 mL

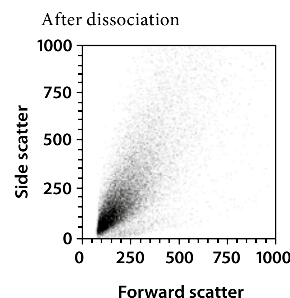
▲ **Note:** In case of very small amount of tissue (<100 mg) cell debris removal can be performed in a 2 mL tube using 300 µL of the Debris Removal Solution, 1000 µL of D-PBS for resuspension of the cell suspension, and ~1000 µL D-PBS for overlay.

1. Centrifuge the cell suspension at 300×g for 10 minutes at 4 °C.
2. Aspirate supernatant completely.
3. Resuspend cell suspension carefully with the appropriate volume of cold D-PBS according to the table above and transfer cell suspension to a 15 mL tube. Do not vortex.
4. Add appropriate volume of cold Debris Removal Solution.
5. Mix well by pipetting 10 times slowly up and down using a 5 mL pipette.
6. Overlay very gently with 4 mL of cold D-PBS.
   ▲ **Note:** Tilt tube and pipette very slowly to ensure that the D-PBS phase overlays the cell suspension and phases are not mixed.
7. Centrifuge at 4 °C and 3000×g for 10 minutes with full acceleration and full brake. Three phases are formed.
8. Aspirate the two top phases completely and discard them.
9. Fill up with cold D-PBS to a final volume of 15 mL.
10. Gently invert the tube three times. Do not vortex!
11. Centrifuge at 4 °C and 1000×g for 10 minutes with full acceleration and full brake.
12. Aspirate supernatant completely.
13. Resuspend cells carefully in the appropriate buffer or medium by pipetting slowly up and down. Do not vortex!
14. (Optional) If the cell pellet contains a high amount of erythrocytes it is recommended to remove them by using the Red Blood Cell Lysis Solution.
15. Cells should be processed immediately for further applications.

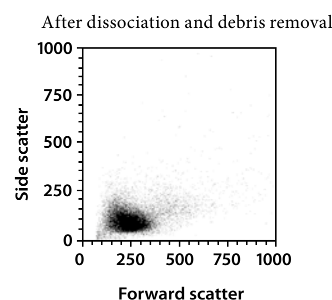
3. Example of debris removal after adult rat heart dissociation using the Debris Removal Solution

One total adult rat heart was dissociated using the Neonatal Heart Dissociation Kit, mouse and rat in combination with the gentleMACS™ Octo Dissociator with Heaters. Subsequently, debris was depleted using the Debris Removal Solution and erythrocytes were removed according to the red blood cell lysis protocol described in the Neonatal Heart Dissociation Kit data sheet. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer based on scatter signals to demonstrate absence of debris after debris removal.

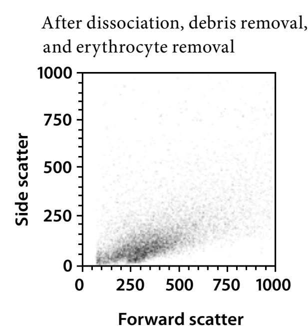
After dissociation



After dissociation and debris removal



After dissociation, debris removal, and erythrocyte removal



Refer to **www.miltenyibiotec.com** for all data sheets and protocols.

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