# **High Efficiency Debris Removal Kit Instructions**

#### **Product Information**

Product Name	Model	Specification
High Efficiency Debris Removal Kit	DHDR -5006	50 T
High Efficiency Debris Removal Kit (Trial Pack)	DHDR -10	8 T

## Description

The High Efficiency Debris Removal Kit (Kit) is a downstream application of the enzymatic digestion kit, which is used to remove debris from single cell suspension, to obtain clean cell suspension with clean background and less debris. The Kit is applied in removal of cell debris of single cell suspension from adult mouse and rat brain tissue, adult mouse heart tissue, adult mouse liver tissue and other kinds of tissues. The prepared single cells can be used to conduct downstream experiments, such as primary cell culture, flow analysis, single-cell sequencing.

#### Components

Product Name	Components	Specification	Storage Condition
High Efficiency Debris Removal Kit	High Efficiency Debris Removal Kit (solution)	2 vial	2°C ~ 8°C
High Efficiency Debris Removal Kit (Trial Pack)	High Efficiency Debris Removal Kit (solution)	1 vial	2°C ~ 8°C

## **Test Capacity**

Recommendation for single process of tissue:

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Tissue Type	Capacity	Capacity-Trial Pack	Initial Sample Dosage		
	200 T	32 T	Single Cell Suspensions obtained from 50 ~ 100 mg mammalian tissue		
Mammalian Tissue	100 T	16 T	Single Cell Suspensions obtained from 101 ~ 500 mg mammalian tissue		
	50 T	8 T	Single Cell Suspensions obtained from 501 ~ 1000 mg mammalian tissue		

# **Storage & Transportation**

- Transported at  $2^{\circ}$ C ~  $8^{\circ}$ C.
- The Kit is valid for 12 months from the date of manufacture.

## **Reagent & Instrument**

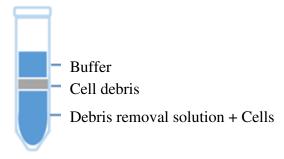
Reagent	D-PBS / PBS
Instrument	High-Speed Benchtop Refrigerated Centrifuge (RWD: # M1416R)

# **Operation**

(1) The weight range of tissue to be processed is 50 mg ~ 1000 mg, refer to the following table for debris removal processing:

Tissue Weight	D-PBS / PBS	<b>Debris Removal Solution</b>	Overlay (D-PBS / PBS)	Reagent Tube
50 ~ 100 mg	1550 μL	450 μL	2 mL	15 mL Centrifuge Tube
101 ~ 500 mg	3100 μL	900 μL	4 mL	15 mL Centrifuge Tube
501 ~ 1000 mg	6200 μL	1800 μL	4 mL	15 mL Centrifuge Tube

- (2) According to the tissue weight range, add corresponding D-PBS or PBS to resuspend the cell pellet from tissue (aspirate as much supernatant as possible and do not shake the suspension), add corresponding volume of debris efficient removal reagent (use a 1 mL pipette gently blow 10 times to blend the cell suspension) and the upper D-PBS or PBS volume (slowly add pre-cooled D-PBS or PBS along the wall of the centrifuge tube).
- (3) Then, centrifugate the cell suspension at 3000×g at 4°C, with an acceleration of 9 and a deceleration of 3 for 10 min. After the centrifugation, the solution is separated into 3 layers (as shown below). Discard the top two layers discarded, collect the cells of lower layer and add cold D-PBS or PBS solution to make sure the total volume of the solution is 10 mL. Invert up and down 3 times (do not shake the suspension), centrifugate the cell suspension at 1000×g for 10 minutes and discard the supernatant completely.



(4) Resuspend the cells with appropriate buffer or medium to required volume for subsequent experiments.

## Precautions

- (1) The Kit is valid for 12 months, and RWD shall not guarantee the validity of expired products.
- (2) When adding the upper layer buffer, the buffer needs to be pre-cooled in advance and slowly added to the top of the cell suspension along the wall of the centrifuge tube.
- (3) In the centrifugation step of removing debris, the speed of acceleration and deceleration is recommended to be 9 up and 3 down, mainly applicable to Eppendorf and Thermo Fisher centrifuges. Other brands of centrifuges can refer to this speed for pre-experiment to determine a more appropriate speed of acceleration and deceleration.
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