High Activity Umbilical Cord Enzymatic Digestion Kit (Human) Instructions

Product Information

Product Name	Model	Specification
High Activity Umbilical Cord Enzymatic Digestion Kit (Human)	DHUTE-2516	25 T

Product Description

High Activity Umbilical Cord Enzymatic Digestion Kit (Human) (The "Kit") can gently, quickly, and efficiently prepare umbilical cord tissues into single cell suspensions. This optimized protocol can help obtain as many single cell samples with high cell viability as possible, while preserving the important surface antigenic epitopes of the cells. The single cell suspensions can be used for further experimental procedures, such as cell sorting, umbilical cord-derived mesenchymal stem cell culture and single cell sequencing.

Main principle: The umbilical cord tissues are prepared by a combination of mechanical shearing and enzymatic digestion of the extracellular matrix (while maintaining the integrity of tissue structure). RWD Single Cell Suspension Dissociator primarily plays a role in mechanical dissociation, while the Kit mainly digests the tissues through enzymatic dissociation. After dissociation, the tissues are filtered through the cell strainer to remove tissue debris to obtain single cell suspensions.

Components

Product Name	Components	Specification	Storage Condition
High Activity Umbilical Cord Enzymatic Digestion Kit (Human)	Enzyme A reagent (powder)	1 vial	2 ~ 8℃
	Enzyme B reagent (powder)	1 vial	2 ~ 8℃
	Enzyme C reagent (powder)	1 vial	-25 ~ -15℃
	Enzyme D reagent (powder)	1 vial	-25 ~ -15℃
	Buffer H (solution)	2 vial	2 ~ 8℃
	Buffer C (solution)	1 vial	2 ~ 8℃
	Buffer D (solution)	1 vial	2 ~ 8℃

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Initial Sample Dosage
Human Umbilical Cord Tissue	25 T	0.5 ~ 2.0 g to be processed per time

Storage & Transportation

- ✧ Transported at 2 ~ 8℃.
- ✧ The Kit is separated into two packages due to different storage temperature, please store them separately according to the attached temperature label.
- ✧ It is recommended to dissolve the enzyme reagent and mix it well and store it in separate packages. Avoid repeated freezing, thawing and shaking.
- ✧ The Kit is valid for 12 months from the date of manufacture.

Reagent & Instrument

Reagent	HBSS Buffer (with Ca^{2+} and Mg^{2+})	RPMI 1640 or DMEM Medium	PBS Buffer
	Red Blood Cell Lysis Buffer (Optional)		
Consumable	Tissue Processing Tube (RWD)	Heater (RWD # HJ-400)	100μm Cell Strainer
	0.22 μm Syringe Filter (Optional)		
Instrument	Single Cell Suspension Dissociator (RWD)	Vortex Oscillator	Constant Temperature Oscillator

Operation

Preparation

(1) Preparation of enzyme A solution: Dissolve the enzyme A reagent with 8.1 mL HBSS buffer (with Ca^{2+} and Mg^{2+}) and store at -25 ~ -15℃. Avoid repeated freezing, thawing and shaking. The solution can be stably stored for 6 months at -25 ~ -15℃ (centrifuge tube can help dissolve the powder).

(2) Preparation of enzyme B solution: Dissolve the enzyme B reagent with 5.4 mL HBSS buffer (with Ca^{2+} and Mg^{2+}) and store at -25 ~ -15℃ (incubated at 37℃ for 5 min when dissolved). Avoid repeated freezing, thawing and shaking. The solution can be stably stored for 6 months at -25 ~ -15℃.

(3) Preparation of enzyme C solution: Dissolve the enzyme C reagent with 2.7 mL buffer C and store at -25 ~ -15℃. Avoid repeated freezing, thawing and shaking. The solution can be stably stored for 6 months at -25 ~ -15℃.

(4) Preparation of enzyme D solution: Dissolve the enzyme D reagent with 2.7 mL buffer D and store at -25 ~ -15℃. Avoid repeated freezing, thawing and shaking. The solution can be stably stored for 6 months at -25 ~ -15℃.

(5) Preparation of enzyme mixture:
Prepare the enzyme mixture according to the table below, and the mixture should be freshly prepared just before use. The enzyme mixture can be used to process up to 2.0 g umbilical cord tissue. If the weight of tissue to be processed is greater than 2.0 g, more tissue processing tubes are needed; if less than 1.0 g, the amount of the mixture can be halved. For conducting subsequent cell culture, the enzyme mixture should be sterile-filtered with a 0.22 μm syringe filter. After filtration, the total volume of the mixture should be 4 mL.
⚠ Note: Before preparing the enzyme mixture, the enzyme B solution needs to be incubated in a 37℃ constant temperature oscillator for about 5 min until it is completely dissolved.

Enzyme Mixture				
Buffer H 3.3 mL	Enzyme A 300 μL	Enzyme B 200 μL	Enzyme C 100 μL	Enzyme D 100 μL

(6) Activation of enzyme mixture:
Place the tissue processing tube containing the prepared enzyme mixture in the 37℃ constant temperature oscillator and rotate it continuously at 50 ~ 100 rpm for 25 ~ 30 min.

Mechanized Protocol

✧ **Protocol for fresh umbilical cord tissue**

(1) After obtaining fresh umbilical cord tissue or the umbilical cord tissue in the preservation solution, first prepare and activate the enzyme mixture. During activation, it is suggested to pre-treat the tissue.

(2) Cut off both ends of the intact umbilical cord tissue and rinse the rest tissue several times in pre-cooled PBS buffer. Then, cut the tissue into small sections and rinse again to remove as much of the remaining blood as possible. Two methods can be adopted to process the tissue: One is to cut the tissue into 2 ~ 4 mm pieces in a clean petri dish with scissors, the other is to remove two arterial blood vessels and one venous blood vessel by scissors, hemostat or forceps, peel off tissues on "Wharton's Jelly" or "Amnion" and cut the target tissue into 2 ~ 4 mm pieces.

(3) Weigh the tissue and remove the tissue pieces into the tissue processing tube containing activated enzyme mixture.

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(4) Follow the steps in ***Protocol for frozen umbilical cord tissue*** from step (3) to the end.

✧ **Protocol for frozen umbilical cord tissue**

(1) Unfrozen umbilical cord tissue: Before thawing the tissue, prepare and activate the enzyme mixture. During activation, it is suggested to pre-treat the tissue. Place the frozen tube on the 37℃ constant temperature oscillator for thawing. After thawing, transfer the tissue to a 50 mL centrifuge tube filled with RPMI 1640 or DMEM medium at room temperature for temporary storage. The tissue should be submerged in the solution. Invert the tube for 1 min and use a 100 μm cell strainer to collect the tissue pieces.

⚠ Note: The tissue pieces should be around 3 mm in size and the larger ones can be cut on the sieve.

(2) Weigh the tissue and remove the tissue pieces into the tissue processing tube containing activated enzyme mixture.

(3) Tighten the tissue processing tube, turn it upside down and fit into the cannula of single cell suspension dissociator with heater. Then, run the program **H_Cord_Heater_1**.

⚠ Note: Make sure the sample is in the area where the rotor/stator is located.

(4) After the program has finished, remove the tissue processing tube from the single cell suspension dissociator, invert the tube, and short spin for 7 s or centrifuge at 500×g for 10 s to sink the sample tissue to the bottom.

(5) Wet the 100 μm cell strainer with 1 mL RPMI 1640 or DMEM medium. Filter the cell suspension with the cell strainer and collect the suspension to the 50 mL centrifuge tube.

(6) Rinse the tissue processing tube with 10 mL DMEM medium, filter the solution with the 100 μm cell strainer and collect it to the 50 mL centrifuge tube mentioned in step (5). To clearly observe the cell sediment, the solution can be transferred to a 15 mL centrifuge tube before centrifugation.

(7) Centrifuge the cell suspension at 500×g for 10 min and carefully aspirate all supernatant. Then, resuspend the cell suspension with RPMI 1640 or DMEM medium to required volume for subsequent experiment.

⚠ Note: The number of cells obtained from 1 g of umbilical cord tissue is at the 10^5 level. To increase the cell concentration, the volume of liquid for resuspending the cell sendiment is suggested to be less than 500 μL.

(Optional) Removal of erythrocyte:

If the erythrocyte removal is required, resuspend the cells processed in step (7) with 200 μL red blood cell lysis buffer, place the cells on ice and incubate for 2 ~ 3 min. Then, resuspend with about 1 mL of RPMI 1640 or DMEM medium, centrifuge the cell suspension at 500×g for 5 min and completely discard the supernatant.

Manual Protocol

(1) After gaining tissue sample, prepare and activate the enzyme mixture in a 50 mL centrifuge tube as described in ***Preparation***. Process the tissue as mentioned in ***Mechanized Protocol*** that cuts the tissue into pieces of 2 ~ 4 mm and add the pieces into the centrifuge tube containing enzyme mixture.

(2) Incubate the tissue in the 37℃ constant temperature oscillator at 50 rpm.

(3) Incubate the tissue for a total of about 3 h, during which it is necessary to take out every 40 min or so and oscillate in the vortex oscillator for 20 s (the speed is adjusted to medium speed). Oscillate for 4 ~ 5 times, and during the digestion, observe the condition of tissue to avoid over-digestion.

(4) After digestion, if there is still much residual tissue, oscillate the suspension again on the vortex oscillator. Open the centrifuge tube, use a 1 mL pipette which the tip is cut off about 5 mm to blow and mix the cell suspension 20 times.

(5) Follow the steps in ***Protocol for frozen umbilical cord tissue*** from step (5) to the end. When filtering the tissue, residual tissues can be blew and ground on the sieve by the 1 mL pipette with appropriate amount of medium.

⚠ Note: Compard with mechanized protocol, the manual protocol has a certain fluctuation of cell number and incomplete digestion. It is suggested to extend the time of digestion or increase the times of blowing.

Precautions

(1) The Kit is valid for 12 months and RWD shall not guarantee the validity of expired products.

(2) For any downstream cell culture to be performed subsequent to tissue dissociation, it is necessary to ensure that all steps are performed under sterile conditions.

(3) The number of cells obtained from 1 g of umbilical cord tissue is at the 10^5 level, and a tissue processing tube with 4 mL of enzyme mixture can treat up to 2 g of umbilical cord. If more cells are needed, it is recommended to increase the number of tissue processing tubes, and combine all the cells together.

(4) The Kit has passed the transportation test. The performance of the Kit is not affected though the ice pack equipped with the Kit has melted upon receipt.

* Note: The tissue processing tubes of RWD are not available in the USA.

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Version [A]
Date: 2024.11