High Activity Hepatocyte Extraction Kit (Mouse & Rat) Instruction

1 Product Information

Product Name	Model	Specification
High Activity Hepatocyte Extraction Kit (Mouse & Rat)	DHHE-2515	15 T

2 Description

The High Activity Hepatocyte Extraction Kit (Mouse & Rat) (the "Kit") can prepare liver tissue from mouse or SD rat (6 ~ 9 weeks) into hepatocytes suspension in a mild and efficient manner. This optimized protocol enables more single cell samples with high cell viability and few debris, which can be applied in downstream experiments in terms of primary cell culture, pharmacology, pathogenesis of liver disease of toxicology, hepatocyte transplantation and metabolic disease research model.

Main principle: The liver tissue of mouse and rat are prepared to single cell suspensions with the method of two-step perfusion. RWD single cell suspension dissociator mainly plays a role in mechanical dissociation that more single cells can be mildly released without being damaged, while the Kit is mainly used to digest the tissue by enzymatic dissociation. After dissociation, the sample is filtered with a cell strainer to remove tissue residue that the single cell suspension with high cell viability can be obtained

3 Components

8 vials of reagents in total, including:

1 vial of Enzyme A reagent (powder)
1 vial of Enzyme B reagent (powder)
1 vial of Enzyme C reagent (powder)
1 vial of Buffer B
1 vial of Buffer C
1 vial of reagent E (powder)
1 vial of reagent G (powder)
1 vial of live cell purified solution

4 Test Capacity

15 mouse liver tissue dissociations or 7 SD rat liver tissue dissociations can be performed with 1 mouse or rat being processed each time.

5 Transportation & Storage

Transport at 2℃ ~ 8℃.

The enzyme C should be stored at -25℃ ~ -15℃ and other reagents be stored at 2℃ ~ 8℃ with a shelf life of 12 months.

6 Reagents & Instruments

FBS
PBS buffer
NaOH solution
DMEM or RPMI 1640
HBSS buffer (with Ca^{2+} and Mg^{2+})
Water bath
Peristaltic pump
100 μm cell strainer
Small syringe needle or indwelling needle
Heater (optional, RWD: # HJ-400)
Tissue processing tube* (optional, RWD)
Single cell suspension dissociator (optional, RWD)

7 Methods

7.1 Reagents Preparation

(1) Preparation of enzyme A solution: Dissolve the enzyme A powder with 14 mL HBSS buffer (with Ca^{2+} and Mg^{2+}) and directly store the solution at -25℃ ~ -15℃, avoiding repeated freeze-thaw and shake. Under this condition, the solution can be stored stably for 6 months (it can be dissolved in a 50 mL centrifuge tube).

(2) Preparation of enzyme B solution: Dissolve the enzyme B powder with 3.2 mL Buffer B and directly store the solution at -25℃ ~ -15℃, avoiding repeated freeze-thaw and shake. Under this condition, the solution can be stored stably for 6 months.

(3) Preparation of enzyme C solution: Dissolve the enzyme C powder with 0.8 mL Buffer C and directly store the solution at -25℃ ~ -15℃, avoiding repeated freeze-thaw and shake. Under this condition, the solution can be stored stably for 6 months.

(4) Preparation of Buffer E solution: Dissolve the reagent E powder with 28 mL ultra-pure water and directly store the solution at -25℃ ~ -15℃, avoiding repeated freeze-thaw and shake. Under this condition, the solution can be stored stably for 6 months.

(5) Preparation of Buffer G solution: Dissolve the reagent G powder with 22.4 mL ultra-pure water and directly store the solution at -25℃ ~ -15℃, avoiding repeated freeze-thaw and shake. Under this condition, the solution can be stored stably for 6 months.

7.2 Perfusate Preparation

Prepare the perfusate based on the table below. The perfusate should be ready-to-use and only can process liver tissue of one mouse. If cell culture is necessary, the perfusate should be aseptically filtered with a 0.22 μm filter head, and the volume of each perfusate is 70±1 mL after filtration.

Name	Preparation	PH
Perfusate①	1.75 mL Buffer E + 0.7 mL Buffer G + 67.55 mL PBS	Adjust PH to 7.2 ~ 7.4 (Recommend: add 45 μL 4M NaOH)
Perfusate②	0.875 mL Enzyme A + 0.2 mL Enzyme B + 0.05 mL Enzyme C + 0.7 mL Buffer G + 68.175 mL HBSS buffer (with Ca^{2+} and Mg^{2+})	/

⚠ Note: After the preparation and subpackage of 10 mL perfusate②, put it in a 37°C water bath for use (step (5)), and the rest of the perfusate① and perfusate② also need to be preheated in a 37°C water bath for 30 min before the perfusion experiment.

⚠ Note: If SD rat liver tissue needs to be processed, the volume of perfusate should be doubled, namely 140 mL perfusate① and 140 mL perfusate②.

⚠ Note: The whole centrifugation needs to be at 4°C, please pre-cool the centrifuge in advance.

7.3 Tissue Enzymatic Digestion

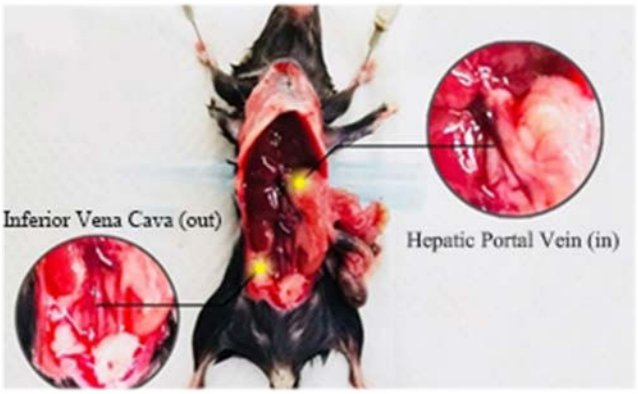
(1) Choose a mouse or rat aged 6 ~ 9 weeks. Anesthetize and fix it, and spray alcohol on its abdomen.

(2) Pick up its abdomen's skin with a forceps and cut off until the whole abdominal cavity is exposed. Fix the inner skin on the both sides with fixing needles to prevent cells from being contaminated by fur.

⚠ Note: Please maintain the integrity of the liver when cutting.

(3) Gently turn the stomach and intestine to the right with the forceps to expose the hepatic portal vein and inferior vena cava. The perfusion tube should be connected to the perfusate① in advance, the air in the whole system is expelled, and the perfusion rate has been set to 5 mL/min. Then insert the injection needle into the hepatic portal vein and start the peristaltic pump. It is demonstrated that the needle is injected into the right position if the liver tends to swell and turn white. Immediately cut the inferiormost edge of the inferior vena cava to release blood and speed up the perfusion rate gradually

to 8 mL/min after the perfusion is stable for 5 ~ 10 s (The maximum perfusion rate of rat is 12 mL/min). It is demonstrated that the perfusion is normal without liquid leakage if the liver tissue is swelling and raising when picking up the inferior vena cava by the forceps.



(4) When the perfusate① is going to be empty (avoid taking in air), stop the pump, replace the perfusate① with 60 mL perfusate② (130 mL for SD rat) and keep the perfusion rate of 8 mL/min (The maximum perfusion rate of rat is 12 mL/min); During perfusion, picking up the inferior vena cava appropriately is allowed to observe whether the liquid leakage occurs. If occurs, the position of the injector should be adjusted in time.

⚠ Note: No bubbles are allowed to enter the perfusion tube when changing the perfusate that the perfusates should be connected closely.

(5) When the perfusion is completed, remove the whole liver tissue to a plate with 5 mL perfusate② and tear the tissue in to pieces in order to release single cells. Add remaining tissues into a tissue processing tube with 3 ~ 5 mL perfusate②, attach the tube into the sleeve of the single cell suspension dissociator, attach the heater and run the program **M_Hepatocyte_Heater**.

⚠ Note: If primary cell culture is necessary, make sure the liver is complete without any damage after the perfusion. In addition, in avoid of contamination in cell culture, rinse the surface of the liver in a medium with 1% double antibody before releasing the single cells.

⚠ Note: Releasing the single cells after the perfusion must be done in the plate or the tissue processing tube with perfusate②. If the is not used, single cell suspension can be obtained by means of tearing or squeezing tissue manually.

(6) After processing the tissue, filter the cell suspension in the plate or the tissue processing tube with a 100 μm cell strainer wet by 1 mL cold DMEM or RPMI 1640 (with 10% FBS). Rinse the plate or the tissue processing tube with another 5 ~ 10 mL cold DMEM or RPMI 1640 (with 10% FBS), filter the liquid with the 100 μm cell strainer and collect the above liquid into a 50 mL centrifuge tube.

(7) Centrifuging: 4℃, 50×g, centrifugate for 3 min. After centrifuging, discard the supernatant with a pipette.

⚠ Note: The cells should be centrifuged at 4℃。

(8) Living cell purification:

(a) Lizvving cell purification dilution = 3 mL live cell purification solution + 5.1 mL cold DMEM or RPMI 1640 (with 10% FBS). The dilution can be used after blending.

(b) If purifying the liver tissue of 1 mouse, add 8 mL living cell purification dilution to resuspend the cell pellzet. Individually add 4 mL of the dilution into two 15 mL centrifuge tubes and carefully add 4 mL DMEM or 1640 medium along the wall of the tubes (Do not disturb the lower layer and visible delamination can be seen after adding DMEM or 1640 medium).

⚠ Note: If more living cells are needed, it is necessary to increase the volume of the living cell purification dilution and divide the dilution into multiple tubes.

(c) Centrifuging: 800×g, set the acceleration to 5 and deceleration to 3 for 10 min at 4℃. After the

centrifuging, extract the suspension (living cell layer) between dilution and medium and transfer it into a new 15 mL centrifuge tube. Add 5 ~ 10 mL cold DMEM or RPMI 1640 (with 10% FBS) and gently blend the dilution upside down.

(d) 50×g, centrifugate for 3 min for 4℃ and discard the supernatant with a pipette after centrifuging.

(e) Use cold DMEM or RPMI 1640 (with 10% FBS) to resuspend the cell to the required volume for follow-up experiment.

8 Precautions

(1) This 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) Enzyme reagents should be stored separately and avoid repeated freezing and thawing. It should be used after dissolving on ice or in a freezer at 4°C to maintain its activity.

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

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