High Activity Hepatocyte Extraction Kit (Mouse & Rat) Instructions

Product Information

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

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 gently, quickly and efficiently. This optimized protocol enables more single cell suspension with high cell viability and few debris, which can be applied in downstream experiments such as 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 suspension with the method of two-step perfusion, which can help release more single cells in a gentle manner and maintain the intactness of the cells. The Kit is used to digest the tissue by enzymatic digestion. After digestion, the cell suspension is filtered through the cell strainer to remove tissue residues to obtain single cell suspension.

Components

Product Name	Components	Specification	Storage Condition
High Activity Hepatocyte Extraction Kit (Mouse & Rat)	Enzyme A Reagent (powder)	1 vial	2℃ ~ 8℃
	Enzyme B Reagent (powder)	1 vial	2℃ ~ 8℃
	Enzyme C Reagent (powder)	1 vial	-25℃ ~ -15℃
	Buffer B (solution)	1 vial	2℃ ~ 8℃
	Buffer C (solution)	1 vial	2℃ ~ 8℃
	Reagent E (powder)	1 vial	2℃ ~ 8℃
	Reagent G (powder)	1 vial	2℃ ~ 8℃
	Living Cell Purified Solution (solution)	1 vial	2℃ ~ 8℃

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Initial Sample Dosage
Mouse Liver Tissue	15 T	A mouse to be processed per time
SD Rat Liver Tissue	7 T	A rat 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 that all the enzyme reagents should be dissolved separately, mixed evenly and stored in small 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+})	PBS Buffer	FBS (Fetal Bovine Serum)
	RPMI 1640 or DMEM Medium	NaOH Solution	
Consumable	Small Syringe Needle or Indwelling Needle	100 μm Cell Strainer	Tissue Processing Tube (optional, RWD)
	Heater (optional, RWD: # HJ-400)	0.22 μm Syringe Filter (optional)	
Instrument	High-Speed Benchtop Refrigerated Centrifuge (RWD: # M1416R)	Constant Temperature Oscillator	Peristaltic Pump
	Single Cell Suspension Dissociator (optional, RWD)		

Operation

Preparation of Reagent

(1) Preparation of enzyme A solution: Dissolve the powder of the enzyme A reagent with 14 mL HBSS (with Ca^{2+} and Mg^{2+}), subpackage the solution and store at -25℃ ~ -15℃. Avoid repeated freezing, thawing and shaking. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃. (The 50 mL centrifuge tube can be used to help dissolve the powder.)

(2) Preparation of enzyme B solution: Dissolve the powder of the enzyme B reagent with 3.2 mL buffer B, subpackage the solution and store at -25℃ ~ -15℃. Avoid repeated freezing, thawing and shaking. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃.

(3) Preparation of enzyme C solution: Dissolve the powder of the enzyme C reagent with 0.8 mL buffer C, subpackage the solution and store at -25℃ ~ -15℃. Avoid repeated freezing, thawing and shaking. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃.

(4) Preparation of Buffer E solution: Dissolve the powder of the reagent E with 28 mL ultrapure water, subpackage the solution and store at -25℃ ~ -15℃. Avoid repeated freezing, thawing and shaking. The solution can be stored stably for 6 months at -25℃ ~ -15℃.

(5) Preparation of Buffer G solution: Dissolve the powder of the reagent G with 22.4 mL ultrapure water, subpackage the solution and store at -25℃ ~ -15℃. Avoid repeated freezing, thawing and shaking. The solution can be stored stably for 6 months at -25℃ ~ -15℃.

Preparation of Perfusate

Prepare the perfusate based on the table below. The perfusate should be prepared just before use and only can be used to process liver tissue of one mouse. If cell culture is necessary, the perfusate should be sterilely filtered with a 0.22 μm syringe filter, and the total volume of filtered perfusate is 70±1 mL.

Name	Preparation	PH
Perfusat ①	1.75 mL Buffer E + 0.7 mL Buffer G + 67.55 mL PBS	Adjust PH to 7.2 ~ 7.4 (Recommendation: add 40 μL 4M NaOH)
Perfusat ②	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, subpackage 10 mL perfusate ② and put it in the 37℃ constant temperature oscillator for use (step (5)). The rest of the perfusate ① and perfusate ② also need to be preheated in the 37℃ constant temperature oscillator for 30 min before perfusion.

⚠ Note: If SD rat liver tissue need to be processed, the volume of perfusate should be doubled, namely 140 mL perfusate ① and 140 mL perfusate ②.

⚠ Note: The whole centrifugation process should be at 4℃, please pre-cool the centrifuge.

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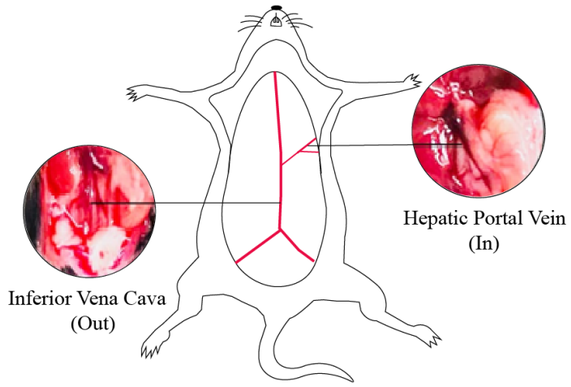
Enzymatic Digestion Protocol

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

(2) Pick up its abdomen's skin with a forceps and cut off the skin to expose the whole abdominal cavity. 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 off the skin.

(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 perfusate ① in advance and the air in the whole system is expelled. Set the perfusion rate to 5 mL/min, insert the syringe needle into the hepatic portal vein and turn on 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 gradually speed up the perfusion rate to 8 mL/min (The maximum perfusion rate of rat is 12 mL/min) after the perfusion is stable for 5 ~ 10 s. It is demonstrated that the perfusion is normal with no liquid leakage when picking up the inferior vena cava by the forceps, the liver tissue is swelling and raising.



(4) When the perfusate ① is going to be empty (avoid taking in air), stop the pump, replace the perfusate ① with 60 mL perfusate ② (130 mL for SD rat) and keep the perfusion rate to 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 liquid leakage occurs. If occurs, the position of the needle should be adjusted.

⚠ Note: No bubbles are allowed to enter the perfusion tube when changing the perfusate, so the perfusate should be replaced in time.

(5) When the perfusion is completed, remove the whole liver tissue to a plate containing 5 mL perfusate ② and gently tear the tissue in to pieces in order to release single cells. Add remaining tissue to the tissue processing tube containing 3 ~ 5 mL perfusate ②, attach the tube in the bushing of the single cell suspension dissociator, mount the heater and run the program **M_Hepatocyte_Heater**.

⚠ Note: If the single cell suspension dissociator is not used, the remaining tissue can be treated by means of tearing or squeezing tissue manually. Releasing single cells after the perfusion must be done in the plate or the tissue processing tube containing perfusate ②.

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

⚠ Note: Releasing single cells after the perfusion must be done in the plate or the tissue processing tube containing perfusate ②. If the single cell suspension dissociator 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 through the 100 μm cell strainer wetted by pre-cooled DMEM or RPMI 1640 medium containing 10% FBS. Rinse the plate and the tissue processing tube with 20 ~ 30 mL cold DMEM or RPMI 1640 medium containing 10% FBS and filter the suspension through the 100 μm cell strainer. Collect the filtered suspension to the 50 mL centrifuge tube and gently turn the tube upside down 5 times.

⚠ Note: If more cells are required, it is allowed to use the forceps to tear off the remaining tissue pieces on the screen of the cell strainer and rinse the cell strainer by medium to gain more cell suspension.

(7) Centrifugation: 4℃, 50×g, centrifugate for 3 min. After the centrifugation is finished, discard the supernatant with a 5 mL pipette.

(8) Resuspend the cell precipitate in step (7) by 20 mL pre-cooled DMEM or RPMI 1640 medium containing 10% FBS. Then, centrifugate the suspension at 4℃, 50×g for 3 min. After the centrifugation is finished, discard the supernatant with the 5 mL pipette.

(9) Purification of living cells:

(a) Living cell purification dilution = 3 mL living cell purification solution + 5.1 mL cold DMEM medium containing 10% FBS. The dilution can be used after blending well.

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

⚠ Note: Please make sure the adding speed is as slow as possible, otherwise the layering effect will be affected.

⚠ 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 more tubes.

(c) Centrifugation: 800×g, acceleration 5 and deceleration 3, 10 min, 4℃. After the centrifugation is finished, extract the suspension (living cell layer) between dilution and medium and transfer it to a new 50 mL centrifuge tube. Add 20 mL cold DMEM or RPMI 1640 medium containing 10% FBS and gently turn the dilution upside down 5 times.

(d) 50×g, centrifugate for 3 min at 4℃ and discard the supernatant with the 5 mL pipette.

(e) Resuspend the cells to required volume for subsequent experiment with pre-cooled cold DMEM or RPMI 1640 medium containing 10% FBS or RPMI 1640 medium or cold buffer containing 0.5% BSA.

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 enzyme reagent should be stored in small packages, and avoid repeated freezing and thawing. It should be used after dissolving on ice or in a refrigerator at 4 ℃ to maintain its activity.

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

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