Kidney Tubular Organoid Growth Medium (Human) Kit

CAT NO.: IKC1022-1
Storage:-20℃， 1 year
Size: 100mL/500mL

Introduction

Kidney Tubular Organoid Growth Medium (Human) Kit contains Human Kidney Tubular Organoid Basal Medium A, 50× Human Kidney Tubular Organoid Supplement B, 250× Human Kidney Tubular Organoid Supplement C. This product can be used to efficiently construct human kidney tubular organoid. The structure and function of the kidney tubular organoid (tubuloids) are similar to that of real kidney tubules. Human Kidney tubular organoid can be used for many applications such as organ development, disease modeling, drug screening, nephrotoxic testing, and regenerative therapy.

Kit Components

Reagent	Size	Storage
Reagent 1 Human Kidney Tubular Organoid Basal Medium A	100mL/500mL	2-8℃
Reagent 2 50×Human Kidney Tubular Organoid Supplement B	2*1mL/10mL	-20℃
Reagent 3 250×Human Kidney Tubular Organoid Supplement C	0.4mL/2*1mL	-20℃

Protocols (*only for reference*)

1. Preparation of Human Kidney Tubular Organoid Complete Medium

Thaw on ice 50×Human Kidney Tubular Organoid Supplement B, 250×Human Kidney Tubular Organoid Supplement C, Prepare Human Kidney Tubular Organoid Complete Medium according to the following groups, mix thoroughly and place on ice for use.

Reagent	10mL	50mL
Reagent 1 Human Kidney Tubular Organoid Basal Medium A	9.76 mL	48.8mL
Reagent 2 50×Human Kidney Tubular Organoid Supplement B	200μL	1mL
Reagent 3 250×Human Kidney Tubular Organoid Supplement C	40μL	200μL

Note：

a. Complete Medium can be stored at 2-8 ° C for up to 2 weeks or -20 ° C for 3 months. Avoid repeated freeze/thaw cycles.
b. It is recommended to thaw the components before the experiment, prepare the complete medium immediately and use it. If it cannot be used up all at once, please pack and freeze it separately.
c. The above operations need to be carried out in a sterile environment.

2. Organoids

1) Resuspend the collected primary tissue cells in Matrigel (this step is recommended to be performed on ice), with a recommended resuspension density of 50-200 tubulars per 50 μ L. It is recommended to resuspend

tumor cells with Matrigel stock solution. If dilution is required, please ensure that the ratio of Matrigel volume to Organoid Medium volume used for dilution is higher than 2:1.

2) Quickly inject the suspension into the bottom of a 24 well cell culture plate, avoiding the formation of bubbles as much as possible, and inject 25-35 μL of suspension into each well. Subsequently, incubate the cell culture plate in a 37°C, 5% CO_2 incubator for 15-25 minutes for solidification.
3) After Matrigel solidified, slowly inject 500μL preheated Human Kidney Tubular Organoid Complete Medium into the edge of each hole to avoid destroying the existing gel structure, and then put the cell culture plate back to 37°C. In a 5% CO_2 incubator.
4) Pay close attention to the growth status of organoids and replace the culture medium every 3 days. Generally, Human Kidney Tubular Organoid generation can be observed within 5-8 days and the first passage can be performed.
5) Suck off the upper layer of culture medium, add 500 μL of pre cooled Human Kidney Tubular Organ Basal Medium A to the well, and use a cell scraper or 1 mL pipette tip to blow to remove the contents of the cell culture well from the culture plate and transfer it to a 1.5 mL EP tube.
6) Use a pipette gun to fully blow and mix until organoids are separated from Matrigel, 250-300 xg, 3min, and collect precipitation. Then add 1 mL of Human Kidney Tubular Organoid Basal Medium A and gently blow thoroughly to disperse the organoids into fragments. If it is difficult to break the organoids into pieces, use 5 times the volume of Organoid digestive fluid to fully digest in 37°C incubator, generally no more than 3 min, then add 1 mL of Human Kidney Tubular Organoid Basal Medium A to terminate digestion.
7) 200-250 xg, 3min, RT. Discard the supernatant and wash 1-2 times with Human Kidney Tubular Organoid Basal Medium A or PBS for later use.
8) Resuspend the collected cells in Matrigel (this step is recommended to be performed on ice), with a recommended resuspension density of 40-200 Tubulars per 50 μL. It is recommended to resuspend tumor cells with Matrigel stock solution. If dilution is required, please ensure that the ratio of Matrigel volume to Organoid Medium volume used for dilution is higher than 2:1.
9) Quickly inject the suspension into the bottom of a 24 well cell culture plate, avoiding the formation of bubbles as much as possible, and inject 25-35 μL of suspension into each well. Subsequently, incubate the cell culture plate in a 37 °C, 5% CO_2 incubator for 15-25 minutes for solidification.
10) After Matrigel solidified, 500μL preheated Human Kidney Tubular Organoid Complete Medium was slowly injected into the edge of each hole to avoid destroying the existing gel structure, and then the cell culture plate was placed back in the 37°C， 5% CO_2 incubator.
11) Pay close attention to the growth status of organoids and record the morphology and distribution of Kidney Tubular Organoids in multiple fields of view.

Note

1. During the passage and digestion of organoids, the fragmentation state should be observed at all times, and the digestion should be terminated when small cell clusters (10-50 cells) appear, so as to avoid excessive time affecting the subsequent growth vitality of organoids.
2. For your safety and health, please wear a laboratory coat, disposable gloves and a mask.
3. This product is only for scientific research and is prohibited for human body use. Do not use for medicine, clinical diagnosis or treatment, food and cosmetics.