Human Bladder organoid culture Lee et al 2018 V.1

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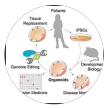

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

HUMAN

GUIDELINES

- Digestion Medium, Basic Medium and Organoids Medium should be freshly prepared.

MATERIALS

Tumor washing

- Organoid culture media
- Hepatocyte media with 10 ng/ml EGF,
- 5% CS-FBS, 10 mM Y-27632 (STEMCELL Technologies),
- 1X Glutamax (GIBCO)
- 100 mg/ml Primocin.

Tumor digestion

- 10 ml of Organoid culture medium
- Hepatocyte media with 10 ng/ml EGF,
- 5% CS-FBS, 10 mM Y-27632 (STEMCELL Technologies),
- 1X Glutamax (GIBCO)
- 100 mg/ml Primocin
- 1/10 Collagenase/hyaluronidase (STEMCELL Technologies).
- 5 mL of TrypLE Express (Invitrogen)
- 10 mL modified Hank's balanced salt solution (HBSS; STEMCELL Technologies)
- 5% CS-FBS
- 10 mM of the ROCK inhibitor Y-27632
- 10 mL of HBSS
- 100 mmcell strainer (Corning)
- Pre-coated 6 well plate (Corning)
- Matrigel (Corning)

Medium Exchange and Splitting

- 1 mg/ml dispase (STEMCELL Technologies)
- PBS IX
- 5 mL TrypLE Express (Invitrogen)

Freezing

- Freezing Medium: 90% CS-FBS and 10% DMSO
- Liquid nitrogen

Washing medium: Organoid culture media (hepatocyte media with 10 ng/ml EGF, 5% CS-FBS, 10 mM Y-27632 (STEMCELL Technologies), and 1X Glutamax (GIBCO)),supplemented with 100 mg/ml Primocin.

Organoid culture medium: (hepatocyte media with 10 ng/ml EGF, 5% CS-FBS, 10 mM Y-27632 (STEMCELL Technologies), and 1X Glutamax (GIBCO)),supplemented with 100 mg/ml Primocin.

Digestion medium: 10 ml of Organoid culture media supplemented with 100 mg/ml Primocin and 1:10 dilution of collagenase/hyaluronidase (STEMCELL Technologies).

Trypsinization stopp medium: 10 mL modified Hank's balanced salt solution (HBSS; STEMCELL Technologies) supplemented with 5% CS-FBS, 10 mM of the ROCK inhibitor Y-27632 and 100 mg/ml Primocin.

Freezing medium: 90% CS-FBS and 10% DMSO

Tumor Washing

1 For tissue dissociation, tumor tissues from patients or xenografts were washed in organoid culture media (hepatocyte media with 10 ng/ml EGF, 5% CS-FBS, 10 mM Y-27632 (STEMCELL Technologies), and 1X Glutamax (GIBCO)),supplemented with 100 mg/ml Primocin, and minced with scissors.

Tumor digestion

2 Tumor tissues were then incubated in 10 mL of the organoid culture media supplemented with 100 mg/ml Primocin and 1:10 dilution of collagenase/hyaluronidase (STEMCELL Technologies) at 37 C for 15 min. Dissociated tissues were spun down at 350 g for 5 min, resuspended in 10 mL of PBS, and spun down again.

The tissues were resuspended in 5 mL of TrypLE Express (Invitrogen), followed by incubation at room temperature for 3 min. Trypsinization was stopped by addition of 10 mL modified Hank's balanced salt solution (HBSS; STEMCELL Technologies) supplemented with 5% CS-FBS, 10 mM of the ROCK inhibitor Y-27632 and 100 mg/ml Primocin, followed by centrifugation at 350 g.

Dissociated tissues were resuspended in 10 mL of HBSS supplemented with5%CS-FBS, 10 mM Y 27632 and 100 mg/ml Primocin, and pass the minced tissue through a 100 mm cell strainer(Corning).

Seeding in matrigel

3 Dissociated cell clusters (approximately 2-10 cells per cluster, and 13106 cells in total) were spun down and resuspended in 60% Matrigel (Corning)/organoid culture media, and plated in a 250 mL drop in the middle of one well of a pre-coated 6-well plate (Corning) with 60% Matrigel. The drop was solidified by a 30-minute incubation at 37 C and 5% CO2.

After solid drops formed, 1.5 mL of the organoid culture media was added to the well, and the medium was changed every 3-4 days. Typically, approximately 50%–80% of the cell clusters would form organoids, although there was considerable variation between lines and not all organoids could propagate after passaging.

Medium exchange and splitting

4 For passaging, 1 mg/ml dispase (STEMCELL Technologies) was added to the medium followed by incubation for 60 min at 37 C to digest the Matrigel. Subsequently, organoids were centrifuged at 350 g for 5 min, washed in PBS and spun down. 5 mL TrypLE Express (Invitrogen) was added, and organoids were incubated at room temperature for 3 min, followed by mechanical dissociation to small cell clusters by pipetting. Organoids were passaged at a 1:2-3 dilution every 2–3 weeks .

Freezing medium

5 To generate stocks, organoids were frozen in 90% CS-FBS and 10% DMSO and stored in liquid nitrogen. Cryopreserved stocks have been successfully recovered for up to at least 18 months after freezing.