Embedding organoids in agarose V.1

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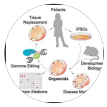

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VERSION 1

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OPEN ACCESS

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Protocol status: In development
We are still developing and optimizing this protocol

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ABSTRACT

This protocol is for embedding organoids in low melting point agarose, the protocol was adapted from Fendler, A., et al. Research Square (2020).

GUIDELINES

- Before starting the protocol, prepare the following solutions 1% BSA in PBS(w/v), 0.1% BSA in PBS(w/v), 10% NBF in PBS(v/v)
- This protocol include one overningt incubation

MATERIALS

Reagents:

- 1.5% of low point melt agarose
- PBS 1x with 1% BSA (v/w)
- PBS 1x with 0.1% BSA (v/w)
- 10% NBF in PBS (v/v)
- 70% EtOH (v/v)
- Embbeding cassette

BEFORE START INSTRUCTIONS

Before starting the protocol, prepare the following solutions and material:

- 0.1% BSA in PBS(w/v),
- 10% NBF in PBS(v/v)
- 1% BSA in PBS.
- Work with 1% BSA/PBS coated tubes and pipetts
- Precool centrifuge
- Prepare the 80°C heating block the day of the agarose embedding

Keywords: Bladder, Embedding, Organoids, Agasore

Organoids embedding in agarose

20m

1 Sample description, Table 1.

	Date	Sample ID	Drops of Matrigel/Wells/Type plate/Passage/Medium

Table 1.

2 Remove the supernatan from the well and add 1 mL cool 1% BSA in PBS to release the organoids from the matrigel, 500 x g, 4°C, 00:04:00 4m

3 Wash the organoids with 1 mL 0.1% BSA in PBS (w/v) , and 300 x g, 4°C, 00:04:00 and discard the supernatant. 4m

4 Resuspend the organoids in 1 mL 0f 10% NBF in PBS (v/v) and fix over night at 4°C rocking.

5 Wash the organoids with 1 mL 0.1% BSA in PBS (w/v) , and 300 x g, 4°C, 00:05:00 and discard the supernatant. 5m
resuspend in 1 mL 0.1% BSA in PBS (w/v)

6 **PAUSE POINT:** The Organois can remain in 0.1% of BSA for a month.

7 4m

300 x g, 4°C, 00:04:00 and discard the supernatant.

8 Shortly place the tube in an
80 °C heating block before carefully overlaying the organoids pellet with
400 µL 1.5% low melting point agarose .

9 300 x g, 4°C let the agarose solidify on ice.

10 After the agarose has polymerized, release the agarose bead from the Eppendorf tube, Use a razor blade to cut off the bottom of the Eppendorf tube at the opposite side as the organoid pellet, and carefully push out the agarose bead.

11 Trim the bead around the organoid pellet, transfer it to an embedding cassette, and place the cassette in
[M] 70 % volume EtoH for 24-48 h.

12 The agarose bead can remain in 70% EtOH for up to 7 days before dehydration.

13 Next step go to the following protocol: Organoids dehydratation and Organoids embedding in paraffin

14

Note

Observations: