



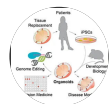
SEP 08, 2023

🌐 Organoids dehydration and Organoids embedding in paraffin

Gabriela Vallejo Flores¹,

Annika Fendler¹

¹Charite



Gabriela Vallejo Flores

OPEN  ACCESS



Protocol Citation: Gabriela Vallejo Flores, Annika Fendler 2023. Organoids dehydration and Organoids embedding in paraffin. **protocols.io** <https://protocols.io/view/organoids-dehydration-and-organoids-embedding-in-paraffin-cy3xyypn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Aug 23, 2023

Last Modified: Sep 08, 2023

PROTOCOL integer ID:
86871

ABSTRACT

This protocol is for Organoids dehydration and Organoids embedding adapted from Fendler, A., et al. Research Square (2020).

GUIDELINES

Before starting the protocol, prepare the following solutions:

- 80% EtOH
- 90% EtOH
- 96% EtOH

MATERIALS

- 80% EtOH
- 90% EtOH
- 96% EtOH
- 100% EtOH
- Xylene
- Paraffin
- Glass staining dish with cover
- Stainless steel rack
- Microtome
- oven

BEFORE START INSTRUCTIONS

- Melt the paraffin at 65 °C during 3-6 hrs in the oven.
- Fill the Glass bucket with the solutions on step 1.
- During the first paraffin incubation time turn on the cooling plate.

Keywords: Organoids,
Dehydration, Embedding,
Paraffin

Dehydration

1 Table 1. Dehydration steps of organoids in agarose beads

	Incubation Time	Solution	C	D
	30 min	80% EtOH		
	30 min	90% EtOH		
	1 hr	96% EtOH		
	1hr	100% EtOH		
	30 min	100% EtOH		
	1h	Xylene		
	30 min	Xylene		
	1-3h	Paraffin		
	Over night	Paraffin		
	1h	Paraffin		

Table 1.

Embedding

- 2 With a forceps, carefully transfer the agarose block into a paraffin embedding jar (metal)
- 3 Carefully add molten Paraffin (65°C), let stand for at least 2 min at 65°C to ensure penetration of paraffin into the agarose block
- 4 Transfer the embedded jar to a cooling plate, and place the plastic embedding cassette on top on the embedding jar (metal), Incubate for 1h to get solid.

5 Specimen are now ready for microtom sectioning with subsequent Immunostaining.

6

Note

After embedding in paraffin, section the paraffin-embedded organoids using a microtome at 5 µm thickness with a water bath at a temperature of 48°C. Quickly transfer the sections onto glass slides to prevent the agarose from dissolving. Let the slides dry for 1 hr at 37°C.

7

Note

Observation: