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

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Deparaffinization for tissue and organoids

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ABSTRACT

This protocol is to withdraw the paraffin from formalin-fixed paraffin-embedded tissue and organoids and to recover the protein structure of your sample.

GUIDELINES

Before star the experiment prepare the following solutions;

- TBS 1x
- Sodium citrate buffer (10 mM Sodium citrate, 0.05% Tween 20, pH 6.0)

Tri-sodium citrate (dihydrate) 2.94 g
Distilled water 1 L
Mix to dissolve. Adjust pH to 6.0 with 1N HCl
Add 0.5 mL Tween 20 and mix well. Store at room temperature for 3 months or at 4°C for longer storage

- Tris-EDTA buffer (10 mM Tris base, 1 mM EDTA solution, 0.05% Tween 20, pH 9.0)

Tris 1.21 g
EDTA 0.37 g
Distilled water 1 L
Mix to dissolve. Adjust pH to 9.0.
Add 0.5 mL of Tween 20 and mix well.
Store at room temperature for 3 months or at 4°C for longer storage

- 96% ETOH
- 80% ETOH
- 70% ETOH

Keywords: Deparaffination, Bladder, Tissue, Organoids

MATERIALS

- TBS 7.6, 100 unit(s), plastic **Roth Catalog #1244.2**
- Sodium citrate tribasic dihydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C8532**
- Tween® 20 **Serva, Germany Catalog #37470**
- Trizma® base **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T6066**
- Ethylenediamine tetraacetic acid disodium salt dihydrate, 250 g **Roth Catalog #8043.1**
- Xylene
- Abs. ETOH
- 96% ETOH
- 80% ETOH
- 70% ETOH
- Distilled water
- Glass staining dish with cover
- Steamer
- Cook heater
- Stainless steel rack

BEFORE START INSTRUCTIONS

- Prepare the steamer and the cook heater for boiling the samples into the antigen retrieval solution
- Fill the Glass bucket with the solutions on step 1

Deparaffinization

1

	Incubation time	Solution	Immersion in the bucket
	10 min	Xylene	

	Incubation time	Solution	Immersion in the bucket
	10 min	Xylene	
	30 sec	100% EtOH	6 times
	30 sec	100% EtOH	6 times
	30 sec	100% EtOH	6 times
	30 sec	96% EtOH	6 times
	30 sec	80% EtOH	6 times
	30 sec	70% EtOH	6 times
	2 min	Distilled water	

Antigen retrieval solution

2 Transfer the slide into the antigen retrieval solution in the steamer, turn on the cook heater and incubate 5 min after the valve is up, turn off the cook heater and wait till the valve get down again.

3 Inmmerse the steamer in running water for 5 min to help the steamer get cooler, open the steamer and add running water to the slides during one min.

4 Transfer the slides into destillated water, now the slides are ready for immunostaining.

5

Note

Observations: