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Processing murine tissues into sections

PLOS One

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ABSTRACT

Processing murine tissues into paraffin sections.

MATERIALS

STEP MATERIALS

⊗ Superfrost Plus glass slides **Contributed by users Catalog #8613967**

⊗ Egg albumin in 50% glycerol **VWR International Catalog #641183**

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SAFETY WARNINGS

- ! -The hydration and rehydration steps and the mounting of the slides are done in a fume hood due to xylene. Exposure to xylene can occur via inhalation, eyes, ingestion or skin contact. Xylene causes health effects from both acute (<14 days) and chronic (>365 days) exposure. In addition, the health effect will be different depending on the amount a person has been exposed to. Therefore, to avoid exposure to xylene via the skin, special xylene-resistant gloves are worn during xylene handling.

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- 1 Fix tissues for 24 hours using 4% formaldehyde in embedding cassettes

- 2 Wash 3 x 15 min with 1x phosphate buffered saline (PBS)

*10x PBS:
-80g NaCl
-2g KCl
-14.4g Na₂HPO₄
-2.4g KH₂PO₄
-Adjust to pH 7.4
-Adjust to 1L with MilliQ water

*1x PBS:
-100 ml 10x PBS
-900 ml denatured water (AD)

- 3 Put cassettes in 70% ethanol and store at +4°C until further processing

- 4 Process cassettes using the Leica TP1020 Tissue Processor (Leica Microsystems):
-70% ethanol 1hr
-80% ethanol 1hr
-99% ethanol 1hr x2
-99% ethanol 2hrs
-100% absolute ethanol 2hrs
-100% ethanol (50%) / 100% xylene (50%) 2hrs
-Xylene 2hrs
-Xylene 3hrs
-Paraffin 3hrs x2

- 5 Embed paraffin-embedded tissues on cassette using metal molds using the Leica EG 1150 H & C Embedding Center

- 6 Cut 8 µm tissue sections using the HM366S microtome (Thermo Fisher Scientific) on albumin-coated Superfrost Plus glass slides.

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