

Processing murine tissues into sections

Bianca Hemmeryckx, Dries Bauters, H. Roger Lijnen

Abstract

Processing murine tissues into paraffin sections.

Citation: Bianca Hemmeryckx, Dries Bauters, H. Roger Lijnen Processing murine tissues into sections. **protocols.io**
dx.doi.org/10.17504/protocols.io.katcsen

Published: 14 Oct 2017

Protocol

Step 1.

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

Step 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)

Step 3.

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

Step 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

Step 5.

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

Step 6.

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



REAGENTS



Superfrost Plus glass slides 8613967 by Contributed by users

Egg albumin in 50% glycerol 641183 by [Vwr](#)

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.