Paraffin Embedding and Sectioning of LunaX™ Hydrogel Constructs

For Research Use Only

Introduction

This protocol describes paraffin embedding and sectioning of LunaX hydrogel constructs, adapted from standard tissue histology methods. It covers fixation, dehydration, clearing, paraffin infiltration, embedding and sectioning, and is optimised to preserve hydrogel structure for downstream histological staining, special stains and immunohistochemical analysis.

Applicable to LunaX hydrogel constructs requiring paraffin-embedded sectioning for histological analysis (e.g. H&E staining, IHC, or special stains). For cryosectioning, refer to LX-APR-007. For whole-mount immunofluorescence, refer to LX-APR-006.

Expected Results

Properly processed LunaX hydrogel constructs yield intact paraffin-embedded sections with preserved construct architecture and minimal tearing or wrinkling, and reliable adherence to positively charged slides after drying. Sections are suitable for downstream histological staining, special stains or immunohistochemistry.

Materials and Equipment

Reagent / Item	Specification / Concentration
Phosphate-buffered saline (PBS)	pH 7.4
Paraformaldehyde (PFA)	4% in PBS; for fixation
Graded ethanol series	30%, 50%, 70%, 90%, 100%
Xylene or xylene substitute	e.g. Histo-Clear; handle in fume hood
Paraffin wax	Melting point 56–58 °C
Embedding molds	Sized appropriately for the construct
Positively charged microscope slides	-
Fume hood	For xylene, PFA and clearing agent handling
Vacuum desiccator	-
Incubator	60 °C
Rotary microtome	-
Water bath	40–45 °C, for section flotation
Slide drying oven	60 °C

Procedure

The procedure below provides recommended starting conditions for paraffin embedding and sectioning of LunaX hydrogel constructs. Users should optimise processing times, reagent compatibility and section thickness according to construct size, matrix composition and downstream staining requirements.

1. Fixation

1. In a fume hood, immerse LunaX hydrogel constructs in 4% PFA in PBS.
2. Incubate at room temperature for 2 hours.
3. Wash thoroughly with PBS (3 × 5 minutes) to remove residual fixative.

> ***Note:*** *Applying vacuum during fixation enhances PFA penetration into the hydrogel matrix but is not a necessity.*

2. Dehydration

1. Transfer constructs to 30% ethanol and incubate for 1 hour at room temperature.
2. Transfer sequentially through 50%, 70%, 90%, and 100% ethanol, 1 hour each.
3. Repeat the 100% ethanol step once more to ensure complete dehydration.

> ***Note:*** *Incomplete dehydration or clearing may reduce paraffin infiltration and lead to section tearing, poor morphology or separation from the paraffin block.*

3. Clearing

1. Transfer constructs to a 1:1 mixture of ethanol and xylene. Incubate for 1 hour.
2. Transfer to 100% xylene and incubate for 1 hour.
3. Repeat the 100% xylene step once more to ensure thorough clearing.

> ***Note:*** *All xylene and xylene-substitute steps must be performed in a fume hood.*

4. Paraffin Infiltration

1. Transfer constructs to a 1:1 mixture of xylene and molten paraffin at 60 °C for 1 hour.
2. Move to 100% molten paraffin at 60 °C for 1 hour.
3. Repeat 100% paraffin infiltration twice more to ensure complete wax penetration.

> ***Note:*** *Optional – Applying vacuum during paraffin infiltration steps can significantly improve wax penetration into LunaX hydrogel constructs.*

5. Embedding

1. Place paraffin-infiltrated constructs into embedding molds filled with molten paraffin.
2. Orient samples appropriately for the planned sectioning plane and allow the paraffin to solidify at room temperature.
3. Cool further on a cold plate or in a refrigerator until fully hardened.

6. Sectioning

1. Trim the paraffin block to expose the sample face.
2. Section using a rotary microtome at 5–10 μm thickness, depending on the intended application.
3. Float sections on a 40–45 °C water bath to remove wrinkles and flatten the section.
4. Mount sections onto positively charged microscope slides.

7. Drying

1. Place slides in a slide drying oven at 60 °C for 30–60 minutes to ensure section adhesion before downstream staining.

Notes

- Dehydration, clearing and paraffin infiltration steps are listed as 1 hour each. Larger constructs may require longer dehydration, clearing and paraffin infiltration times to ensure complete reagent exchange.
- Handle LunaX hydrogel constructs gently throughout the protocol to maintain structural integrity.
- If using xylene substitutes, verify compatibility with LunaX ECM chemistry and with all planned downstream staining applications before committing samples.
- Adjust section thickness to suit the imaging or staining application (e.g. 5 μm for IHC; 5–10 μm for H&E staining).
- This protocol has been adapted from standard tissue paraffin embedding and optimised for LunaX hydrogel constructs.

References

- LX-APR-006 – Whole-Mount Immunofluorescence Staining of LunaX Hydrogel Constructs (Gelomics)
- LX-APR-007 – Staining and Imaging of Cryosectioned LunaX Hydrogel Constructs (Gelomics)
- Protocols and manufacturers' instructions for downstream stains, antibodies and other staining reagents.