Staining and Imaging of Cryosectioned LunaX™ Hydrogel Constructs

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Introduction

This protocol describes the process for staining and imaging of cryosectioned LunaX hydrogel constructs, using histological, histochemical, immunohistochemical or immunofluorescence methods. It enables high-resolution imaging of target proteins, cellular structures and extracellular matrix components in sectioned constructs.

This protocol is applicable to fixed LunaX hydrogel constructs embedded in OCT compound and cryosectioned. For whole-mount staining of intact constructs, refer to LX-APR-006. For paraffin-embedded sections, refer to LX-APR-008.

Expected Results

Correctly processed cryosections retain construct morphology and are suitable for histological, histochemical, immunohistochemical or immunofluorescence staining. Histological and histochemical stains show appropriate contrast for the target tissue feature or matrix component.

Antibody-based staining shows specific labelling of target proteins, cellular structures or extracellular matrix components, with nuclear and, where included, cytoskeletal counterstains clearly resolved. For antibody-based staining, isotype, no-primary-antibody and secondary-only controls show minimal non-specific signal.

Materials and Equipment

Reagent / Item	Specification / Concentration
Paraformaldehyde (PFA)	4% solution in PBS for fixation; handle in a fume hood
OCT compound	Cryosectioning embedding medium
Ice-cold acetone	For section fixation
Tris-HCl buffer	100 mM, pH 7.3
$BaCl_2$ solution	50 mM in 100 mM Tris-HCl, pH 7.3
Phosphate-buffered saline (PBS)	pH 7.4
Bovine serum albumin (BSA)	2% in PBS, or appropriate serum for blocking
Histological staining reagents	As per manufacturer's recommendation
Primary antibodies	Diluted in PBS + 2% BSA or serum
Secondary antibodies + DAPI	Secondary antibodies diluted in PBS + 2% BSA or serum; DAPI at 5 μg/mL
Phalloidin (optional)	Fluorophore-conjugated; for F-actin/cytoskeletal staining; 1:1,500 dilution
Histological or histochemical staining reagents	As per manufacturer's recommendation

Reagent / Item	Specification / Concentration
Antigen retrieval buffer (optional)	As required for target antigen and antibody
Endogenous enzyme blocking reagent	E.g. hydrogen peroxide block for HRP-based detection
Immunohistochemistry detection reagent	HRP-, AP-, polymer- or biotin-based system, as appropriate
Chromogenic substrate	E.g. DAB or alkaline phosphatase substrate
Mounting medium	E.g. ProLong™ Gold or equivalent hard-set medium
Fume hood	For PFA preparation, handling and disposal
Positively charged microscope slides	-
Coverslips	-
Cryostat	-
Humidified staining chamber	-
Brightfield or Fluorescence microscope	Widefield or confocal, with appropriate filter sets
Image analysis software	e.g. ImageJ/Fiji

Procedure

This procedure provides recommended starting conditions and an overview of the general workflow for cryosectioning, staining and imaging of LunaX constructs. Users should optimise staining parameters according to the staining method, target antigen, antibody, construct size and cell type, with reference to relevant manufacturer's instructions and published methods where applicable.

1. Fixation of Hydrogel Constructs

1. In a fume hood, add 4% PFA (0.5–1 mL depending on well size) and incubate for 2 hours at room temperature.
2. In a fume hood, wash thoroughly with PBS (3 × 5 minutes) to remove residual fixative.

2. Embedding and Cryosectioning

1. Fill a cryomold with OCT compound. Embed fixed constructs in OCT compound and freeze on dry ice or in liquid nitrogen. Aim to position the construct centrally within the OCT compound.
2. Section at 10 μm thickness using a cryostat (or your preferred alternative thickness).
3. Mount sections onto positively charged microscope slides.
4. Air-dry sections for 10–15 minutes at room temperature.

3. Section Fixation

1. Fix cryosections with ice-cold acetone for 10 minutes.
2. Air-dry sections completely at room temperature before proceeding.

4. Rehydration and Buffer Conditioning

1. Rehydrate sections in 50 mM $BaCl_2$ in 100 mM Tris-HCl buffer, pH 7.3, for 1 hour at room temperature.

   > ***Note:*** *The $BaCl_2$ / Tris-HCl rehydration step is important for LunaX hydrogel cryosections, as it helps restore section integrity and antigen accessibility.*

2. Rinse briefly in 100 mM Tris-HCl buffer (pH 7.3) for 5 minutes.

5. Histological Staining

1. For histological or histochemical staining, select an appropriate stain for the target tissue feature, such as H&E for general morphology, Alcian Blue for glycosaminoglycans, or Alizarin Red for mineral deposition.
2. Prepare and apply the selected stain according to the manufacturer's instructions, institutional protocols, or established published methods.
3. Optimise staining conditions, including incubation time, rinse steps, differentiation, counterstaining and mounting, according to the stain, section thickness and construct composition.

6. Blocking

1. For antibody-based methods, incubate sections in PBS containing 2% BSA or appropriate serum for 30–60 minutes at room temperature to block non-specific binding.

7. Immunohistochemistry Staining

1. For chromogenic immunohistochemistry, perform antigen retrieval, endogenous enzyme blocking, and serum or protein blocking if required for the target antigen, antibody and detection system.
2. Apply the primary antibody in an appropriate antibody diluent and incubate according to the antibody supplier's instructions or established published methods.
3. Apply the appropriate enzyme-conjugated secondary antibody or detection reagent, then develop signal using a compatible chromogenic substrate, such as DAB or an alkaline phosphatase substrate.
4. Counterstain, rinse and mount sections using reagents and mounting media compatible with brightfield imaging.

8. Immunofluorescence – Primary Antibody Incubation

1. For antibody staining, dilute primary antibodies in PBS + 2% BSA or serum and apply to sections in a humidified chamber.
2. Incubate overnight at 4 °C.
3. Wash sections 2× with PBS (5 minutes each).

9. Immunofluorescence – Secondary Antibody and Nuclear Counterstaining

1. Dilute secondary antibodies in PBS + 2% BSA or serum. Add 5 μg/mL DAPI.
2. Incubate for 1 hour at room temperature in the dark.
3. Wash sections 2× with PBS (5 minutes each), protected from light.

10. Mounting and Imaging

1. Remove excess liquid and apply a mounting medium compatible with the selected stain and imaging method.
2. Place coverslips and allow to cure (e.g. overnight for hard-set media).
3. Acquire images using either a brightfield microscope or fluorescence microscope with appropriate filter sets – depending on your staining protocol.

11. Image Analysis

1. For immunofluorescence imaging, use ImageJ/Fiji or equivalent software to measure marker signal intensity, positive area or localisation.
2. Where applicable, normalise marker signal intensity to DAPI signal to account for differences in cell number between sections.
3. For histological stains, quantify stain-positive area or intensity using a brightfield image analysis workflow appropriate for the stain.

Recommended Controls

- For histological or histochemical stains, include unstained sections or known positive-control sections, where available.
- For antibody-based staining, include isotype control antibodies matched to the species and isotype of each primary antibody and known positive-control sections, where available.
- For immunofluorescence, include secondary-only controls to detect non-specific binding of secondary antibodies.

References

- LX-APR-006 – Whole-Mount Immunofluorescence Staining of LunaX Hydrogel Constructs (Gelomics)
- LX-APR-008 – Paraffin Embedding and Sectioning of LunaX Hydrogel Constructs (Gelomics)
- Protocols and suppliers' instructions for antibodies and other staining reagents.