Technical Note

PARAFFIN BLOCK PREPARATION AND SECTIONING

INTRODUCTION

This document provides a guide for preparing paraffin embedded blocks from fixed tissue specimens and producing sections or scrolls for downstream analyses.

FFPE BLOCK PREPARATION

SECTION 1 – FIXATION

A tissue specimen can be fixed with various fixation agents such as 10% neutral buffered formalin (NBF), Methacarn, and Davidson's, etc. A typical NBF fixation protocol is as follows:

NBF fixation protocol:

1) Optional: perfuse animals with NBF to remove excessive blood and to quickly fix tissues to prevent post-morterm physiological changes.
2) Dissect the tissues of interest, cut into 3-4mm thick pieces, then immerse in 10% NBF (minimum 20:1 fixative/tissue volume ratio) and fix for 16-32 hours at ambient temperature (15-25°C).
3) After fixation, rinse the tissues with H2O or 1XPBS for 10-20 min.
4) Replace H2O or 1XPBS with 70% ethanol (minimum 20:1 solution/tissue volume ratio)
5) Tissue samples can be stored in 70% ethanol at 2-8°C for a few days to weeks before processing into paraffin blocks.

SECTION 2 – PARAFFIN PROCESSING AND EMBEDDING

Tissue processing:

Fixed tissues are placed into tissue processing cassettes and processed using an automated tissue processor. A typical processing protocol is as follows:

Reagent	Time	Temperature	P/V
80% Ethanol	45min	35°C	On
90% Ethanol	45min	35°C	On
95% Ethanol	45min	35°C	On
100% Ethanol	30min	35°C	On
100% Ethanol	30min	35°C	On
100% Ethanol	60min	35°C	On
Xylene	30min	35°C	On
Xylene	30min	35°C	On
Xylene	45min	35°C	On
Paraffin (Paraplast Pluls)	30min	59°C	On
Paraffin (Paraplast Pluls)	30min	59°C	On
Paraffin (Paraplast Pluls)	45min	59°C	On

Paraffin block embedding:

Processed tissues are embedded into paraffin blocks on a tissue embedding station with various histology molds.

Block storage:

To best preserve biomarkers, it is recommended that paraffin blocks be stored at 2-8°C. For long term storage, -15 to -5°C is recommended.

SECTION 3 – SECTIONING

Slide sectioning:

To produce paraffin section slides, trim and section blocks on a rotary microtome following the procedure below:

1) Pre-warm a floating water bath containing fresh dH2O. The typical water bath temperature is 37-42°C. However, depending on the paraffin type, the floating temperature may need to be adjusted to 34-45°C.
2) Trim the block to expose tissue to the surface of block.
3) Briefly chill the block face-down on an ice block for 1-10min depending on the block hardness and tissue type.
4) Cut block into a ribbon of sections at desired thickness. The typical section thickness is 5µm. However, 3-8µm sections are sometimes used for special tissue types or staining.
5) Carefully transfer the section ribbon to the pre-warmed water bath and let sections float until completely spread with no wrinkle or folding. Use forceps to gently separated sections. Do not let section float in water bath for longer than needed.
6) Use a SuperFrost Plus slide to capture the floating section and place on a drying rack. A gentle flicking of the slide can help remove excess amount of H2O.
7) Let slides dry overnight (>12hr) at ambient temperature.
8) Store slides at 2-8°C in a sealed bag with desiccants. For long term storage, store slides at -15 to -5°C in a frost-free freezer.

Scroll sectioning:

To produce paraffin scrolls:

1) Wipe the blade, blade holder, and forceps with RNase Away and dry.
2) Trim the block to expose tissue to the surface of block.
3) Cut block into a paraffin scroll at desired thickness, typically 10-20 µm, then use forceps to transfer the scroll to a DNase/RNase free microcentrifuge tube.
4) Store the scrolls at 2-8°C. For long term storage, store scrolls at -15 to -5°C in a frost-free freezer.

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