



PROSTATE CANCER **BIOREPOSITORY NETWORK**

SOP No: 011

Fixing, Processing and Embedding of Tissue Samples in the Cancer Biomarkers
Team- ICR

STANDARD OPERATING PROCEDURE	SOP No. 011 Fixing, Processing and Embedding of Tissue Samples- ICR
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1. PURPOSE

To describe the procedures for fixing, processing and embedding of tissue samples by the Cancer Biomarker (CB) Team at the Institute of Cancer Research. This procedure applies to all the fresh tissue samples received by the CB team for further processing to Formalin fixed paraffin embedded (FFPE) samples. The purpose is to ensure that all samples are fixed, embedded and processed in a uniform manner and in accordance with the principles of GCP and other applicable regulations.

The following SOP for Blood and Cytology Sample processing is followed by the Institute of Cancer Research Network Site of the PCBN ONLY.

2. RESPONSIBILITIES

Authorized personnel processing tissue samples must:

- Comply with the procedure
- Follow all other precautions required for the handling of human blood and tissue samples
- Obey Team Leader who has overall responsibility of the procedure
- All blood and cytology samples received are logged into the Histopathology Sample Entry (HSE) and on the Progeny.

3. HEALTH AND SAFETY

Personnel carrying out this procedure must maintain safe working practices and observe all relevant Environmental Health and Safety (EH&S) guidelines. This includes the appropriate use of Personal Protective Equipment (PPE), and the procedures for waste disposal, disinfection & spill clean-up and biosafety.

4. EQUIPMENT AND MATERIALS

Equipment	Materials
Class II Cabinet Tissue TEK processor Embedding center	10% neutral buffered formalin (NBF) Xylene Industrial Methylated Spirit (IMS) Distilled water EDTA solution Wax



5. PROCEDURES

5.1 Fixation

- The biopsy should be put in a container with 10% neutral buffered formalin (NBF) ideally within one minute of being taken.
- Make sure the sample is totally submerged in the fixative and it is not stuck to the lid or side of the pot.
- Fix for required time:
 - For needle core biopsies fix at room temperature (RT) for 16-24 hours.
 - For skin punch or excision biopsies fix at RT for 24-30 hours.
 - For bone marrow trephine (BMT) biopsies seal the pot with parafilm and fix at room temperature (RT) for 24-30 hours with agitation. For BMTs, after fixing the sample, briefly rinse in distilled water, then place in a container of EDTA solution, seal and incubate for about 48 hours at 37°C. Change EDTA after approximately 24 hours, and then proceed with incubation.
 - For cell pellets fix overnight at 4°C.
- Preparation of EDTA Solution:
 - Dissolve 50 g sodium hydroxide in 3500 mL distilled water (heats up rapidly, keep stirring)
 - Add 500 g EDTA
 - Stir until solution is clear
 - Note: The pH must be checked and adjusted to 7.0 each day the solution is used. EDTA must be used within 6 months otherwise, it must be discarded and a new one should be prepared.

5.2 Processing

- Note: Samples should be processed immediately after completion of fixation. If this is not possible, the samples can alternatively be transferred to 70% ethanol where they shouldn't be left for more than 12 hours.
 - Operate the tissue processor according to EQU/086/.
 - Label the cassette with trial no. and/or progeny number, initials, study id, date taken and time point, if applicable.
 - Using forceps carefully take the sample out of the NBF or EDTA solution pot and wrap it in tissue wrap paper if the sample is small.
 - For cell pellets, tilt the tube to remove the NBF, gently remove the pellet with a spatula and wrap it in tissue wrap.
 - Seal in the labelled plastic cassette and process through to paraffin wax using one of the following processing schedules:
 - For needle core biopsies use program 8.
 - For skin punch, excision biopsies or BMTs use program 1 or 3.
 - For cell pellets use program 5.
 - Note: Different processing schedules are described in EQU/086/.
 - Record the information required by each trial or study (e.g. total fixation time, processing schedule, core length, etc) in the respective spread sheets and/or Progeny.
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5.3 Embedding

- Operate the embedding center according to EQU/087/.
- In the end of the program remove the processing basket with cassettes from the tissue processor and place in the heated chamber in the embedding center. Take out one cassette at a time and place on the flat heated area at the front of the embedding module.
- Carefully open the cassette lid and either remove the piece of tissue or the wrapped biopsy using heat forceps. Take care to replace the forceps into the heated well when cool down, to avoid the tissue sticking to them, and reheat when necessary.
- Unwrap the tissue paper wrap (if using), and choose the correct size mold for the piece of tissue.
- Fill the mold with molten wax and using the pre-heated forceps, transfer the piece of tissue into the wax and orientate carefully.
- Move the mold on to the cool area of the embedding module, making sure that the piece of tissue does not move. Gently hold in place and flatten with the forceps until the wax starts to solidify.
- Carefully place the cassette base on top, making sure that there are no bubbles trapped underneath.
- Either leave the mold on the cold plate or in the freezer until the wax has completely hardened and the block can be removed easily from the mold.
- If the lids are metal, non-disposable, place back into the processing basket and clean them on the processing machine using the cleaning program.
- FFPE blocks are stored at RT, in lockable cabinets, accessible by all the CB team members, until further processing unless other instructions are given.
 - Molecular Characterization: Stored under progeny number in Cabinet 2.
 - 2472/ TOPARP/ SU2C and other prostate studies: Stored under study ID number, TOPARP number or SU2C number in Cabinet 4.
 - Other trials: Store in assigned box in Cabinet 1 until either shipped or further processed.

References (please contact query@prostatebiorepository.org for information regarding these additional documents):

- 1) MET/155/ (Collection and Handling of Fresh Biopsy Samples in the CB Team)
- 2) EQU/086/ (Operation and Maintenance of the Tissue Processor)
- 3) EQU/087/ (Operation and Maintenance of the Embedding Centre)