

 National Biobank Consortium of Taiwan (Web Link)	National Biobank Consortium of Taiwan Standard Operating Procedure	No.: NBCT SOP-008
	Title: NBCT Wax Block and Blank Section Specimen Preparation Process	Version/Total Pages: No. 1.1 / 6 pages
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1. Purpose

The “National Biobank Consortium of Taiwan” (hereinafter referred to as the “Integration Platform”) primarily integrates domestic human biobanks — their data, biospecimens, and related information (hereinafter referred to as “Integration Platform biospecimen data”) — through a cloud-based system, making them available for application and use by medical, clinical, industry, and related biomedical research investigators nationwide. To achieve cross-institutional collaboration, it is necessary to establish consistent quality standards for dispensed biospecimens and consistent clinical data content. To maintain good specimen quality and to ensure consistency in the handling of wax block and blank section specimens, so as to facilitate future related research, this Standard Operating Procedure is hereby established.

2. Scope of Application

This SOP applies to the process of preparing wax block and blank section specimens.

3. Responsibilities

All human biobanks that have joined the Integration Platform, when dispensing paraffin blank section specimens, are recommended to follow this Standard Operating Procedure.

4. Description

This SOP can be divided into two stages

1. Preparation of tissue or bodily fluid wax blocks
2. Preparation of blank section specimens

5. Operating Procedures

5.1 Wax Block Preparation

Equipment:

Tissue processor, embedding machine

Consumables:

10% neutral buffered formalin, absolute ethanol, paraffin wax pellets

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5.1.1 Human Tissue Wax Block

Procedure:

1. Freshly excised tissue should first be immersed in 10 times its volume of neutral 10% buffered formalin and fixed for at least 2-5 hours. Because formalin penetrates tissue at a rate of only about one centimeter, larger pieces of tissue will require longer fixation — up to overnight immersion at most. It is best to begin dehydration processing within 24 hours, and no later than three days.
2. Trim the fresh or formalin-fixed tissue to an appropriate size, which must be smaller than 25x20x4mm³ (the internal length, width, and height of the dedicated embedding cassette), so that it can be placed into the dedicated embedding cassette, and continue immersing it in neutral 10% buffered formalin.
3. Place the tissue-containing embedding cassettes in a tissue processor and process them according to the standard tissue-processing protocol. The final processing step is paraffin infiltration. Subsequently, use a tissue embedding station to embed the tissues and prepare paraffin blocks.

Precautions:

1. Tissue processing and paraffin embedding require specialized equipment and trained technical personnel. It is therefore recommended that these procedures be performed by a hospital pathology laboratory.
2. Fresh tissues should not be fixed in 10% neutral buffered formalin for an excessively long period, as prolonged fixation may compromise tissue quality and adversely affect subsequent immunohistochemical staining or genetic analysis.
3. If tissues fixed in 10% neutral buffered formalin cannot undergo tissue processing and paraffin embedding within 3 days, replace the formalin with an equal volume of 75% ethanol and store the tissues at 4°C until they can be submitted for processing.

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5.1.2 **Wax Block Prepared from Centrifuged Sediment Cells of Pleural or Ascitic Fluid**

Procedure:

1. Collect at least 50 mL of pleural effusion or ascitic fluid in a 50 mL conical centrifuge tube.
2. Centrifuge the pleural effusion or ascitic fluid specimen at 1,500 rpm for 20 minutes.
3. Aspirate and discard the supernatant. Add 15 mL of phosphate-buffered saline (PBS), and gently tap the tube from side to side to thoroughly resuspend the cell pellet.
4. Centrifuge again at 1,500 rpm for 20 minutes. Aspirate and discard the supernatant, add 15 mL of PBS, and gently tap the tube from side to side to thoroughly resuspend the cell pellet.
5. Centrifuge at 2,500 rpm for 20 minutes. Aspirate and discard the PBS supernatant, add 15 mL of 10% neutral buffered formalin, and mix thoroughly. Allow the tube to stand at room temperature for 40 minutes to fix the sedimented cells and facilitate formation of a cohesive cell clot.
6. Centrifuge again at 2,500 rpm for 20 minutes. Aspirate and discard the supernatant containing 10% neutral buffered formalin, and then add 15 mL of 95% ethanol. Do not shake or mix the tube. Store the tube at 4 °C for at least 2 hours to further fix and dehydrate the cell layer.
7. Remove the conical centrifuge tube from the refrigerator. At this stage, the cell layer should have formed a cohesive cell clot. Transfer the cell clot into a tissue-processing cassette and submit it for tissue processing, dehydration, and paraffin embedding to prepare a paraffin-embedded cell block.

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5.2 Blank Section Specimen Preparation

Equipment:

1. Microtome
2. Laboratory oven

Consumables:

1. Empty slide storage boxes (capacity: 100 slides per box)
2. Coated microscope slides
3. Microtome blades

Procedure:

1. Retrieve the paraffin-embedded tissue blocks from cases that meet the applicant's inclusion criteria, and arrange them according to the case identification numbers assigned to the Integrated Platform application.
2. Based on the number of unstained sections requested by the applicant, write the corresponding Integrated Platform case identification number on the white frosted labeling area at the top of each coated slide.
3. Using a microtome, prepare serial sections at a thickness of 4 μ m. Mount each section onto a slide and place the slides sequentially by identification number in a 100-slide storage box.
4. Place the slide box in a laboratory oven at 42°C for 2 days to remove residual moisture. After drying, store the slides at 4°C or -20°C.

Description: Dried slides may be stored at 4°C for short-term storage. For long-term storage, storage at -20°C or -80°C is recommended.

Notes:

1. It is recommended that all unstained sections provided to applicants for research purposes be mounted on coated slides. However, standard uncoated glass slides may be used if the sections are intended solely for hematoxylin and eosin (H&E) staining.
2. Inspect the condition of the microtome blade regularly during sectioning to prevent knife marks or scoring artifacts in the tissue sections.

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6. Quality Standards for Dispensing

The following matters should be noted for blank section specimens dispensed by the Integration Platform:

1. Slides must be completely dried; confirm that there is no residual water droplet between the tissue and the glass on each slide (i.e., no moisture causing the tissue to appear raised).
2. The tissue should lie flat on the glass surface of the section, smooth and free of wrinkles or knife marks.
3. For tumor tissue, or wax block sections from pleural or ascitic fluid, a pathologist should first confirm that the section contains sufficient tumor cells.

Note: Before dispensing, the slides are first 37°C baked in an oven for 2 hours, to ensure the slides are completely dried.