Immunocine Cancer Center

Fresh Tumoral Tissue Preservation for Future RNA and Protein Isolation

When obtaining tumoral tissue for future immunotherapy use, it is important to consider the conditions for preservation and eventual processing. This is especially critical for creating a personalized, double-loaded dendritic cell vaccine, as Immunocine offers. Correct preservation of the biopsied or resected tissue will prevent the degradation of genetic material and molecules that educate and activate the dendritic cells against the target tumors.

In this document, you'll find instructions for the correct preservation of human tumoral tissue when considering eventual immunotherapy. This includes:

- Ribonucleic acid (RNA),
- Proteins,
- Whole tissue for pathology analysis (optional).

Materials needed.

- Sterile tube(s) (capacity: 5-50 mL) containing RNA stabilizing solution (such as RNAlater from Thermo Fisher). The volume of the RNA stabilizing solution in the tube should be **at least five times (5X)** the volume of the tissue preserved. **Keep this tube at room temperature until the tissue specimen is added.**
- Powder-free sterile gloves
- Cooler box, set up with enough icepacks to maintain a temperature of 2-8°C (36.5-46.4°F) while transporting to a refrigerator.
- Optional (only for pathology analysis): sterile tube containing balanced formaldehyde solution. This tube can be kept at room temperature at all times.

All tubes should be labeled with the **patient's full name, date of birth, collection date, type of preservation solution (i.e., RNA stabilization or formaldehyde), and anatomical site** where the tissue was collected. The label information in the tubes should match the "Specimen Identification Sheet" for tissue acceptance by the manufacturing laboratory.

Specimen collection.

1. Identify the tubes to be used for tissue collection. Preferably, the tube that will have the priority for tissue collection will be the RNA stabilization tube.

   For example, if five fine-needle aspiration punches are collected, place 4 in the RNA stabilizing solution and 1 in the formaldehyde-balanced solution-containing tube.

   Important**:** Tissues that have been exposed to formaldehyde should **never** be put in the RNA stabilizing solution.

2. Collect the tissues and aseptically drop them in the RNA-stabilizing solution (or balanced formaldehyde solution if required). Do not touch the inside of the tubes and avoid warming up the contents with the hand.

3. **If tissue samples are exploratory in nature**, then proceed with the following plan:
   → Take 4 tissue samples
   → Cut ¼ to ½ of each sample, put in fixative, and label as 1A, 2A, 3A, 4A
   → Send those for histological / pathological analysis
   → Take the remaining, corresponding tissues and preserve per the rest of this instruction sheet, labeling with the parallel 1B, 2B, 3B, 4B
   → This allows for future alignment of histologically confirmed cancer tissue with useable sample for immunotherapy

4. Refrigerate the RNA-stabilizing solution with the specimens in the cooler box immediately after collection. **Do not freeze**. It can then be kept at 2-8°C (36.5-46.4°F) overnight to allow for complete solution permeation of the tissue.

   If desired, the balanced formaldehyde solution can be also kept cooled down with the specimens.

5. Instructions for storage: **Keep refrigerated at 2-8°C (36.5-46.4°F) for maximum 4 weeks.** Make sure the tube containing the tissue is set up straight and tightly closed. The tissue **must** be submerged in the preservation solution **the entire time**.

6. **If without an RNAlater solution**, the next best thing to do is **Flash Freeze** the tissue. This would include one of the following options:

    a. Putting the tissue in a 15ml conical (or equivalent) and submerging the tube in liquid nitrogen for 1-2min.

    b. Putting the tissue in a 15ml conical (or equivalent) and submerging the tube in a Dye-Ice Ethanol bath.

        i. Option A is better, but option B can work as well, and a Styrofoam container or other appropriate dish would suffice.

        ii. With either option, the tube/tissue is **then stored at -80°C until ready for shipment**, which will include submerging the frozen tissue in **RNAlater Ice** before a shipment.

Specimen Labeling and Communication.

7. Fill out the "Specimen Identification Sheet" and place it in/on a bag containing each tissue collected.

    Use separate bags and Specimen Identification Sheets for different specimens.

8. Communicate with your patient coordinator to coordinate the tissue transportation to the laboratory.

Additional Required Tests

9. **If possible**, run the following blood tests concurrently (or within ±3 weeks of tissue procurement) on the patient, as these results **will be required** for ultimate tissue acceptance and processing:

HIV 1 and 2 (antibodies or NAT)	CMV (IgG **and** IgM)
Hepatitis B (antigen or NAT)	EBV (IgG **and** IgM)
Hepatitis C (antibodies or NAT)	*Treponema pallidum* (IgG and IgM)
Toxoplasma (IgG **and** IgM)	*Trypanosoma cruzi* (IgG)

Immunocine Cancer Center

Specimen Identification Sheet

Patient's information (same as passport information)	
Last name:	
First name:	
Middle name:	
Date of birth (dd/mm/yyyy):	
Specimen's Information / Type of preservation (select one using an X)	
RNA-stabilizing solution	
Balanced formaldehyde solution	
Date of collection:	
Time of collection:	
Details of collection: (Please include anatomical site biopsied, number of fragments obtained, and any relevant information about the tissue)	