

ITN091AI VIBRANT: Renal Biopsy Processing for Single Cell Analyses Standard Operating Procedure

Instructions: Study personnel must be trained and authorized by the site Principal Investigator to perform renal tissue processing. Personnel are required to read and understand the following **ITN091AI VIBRANT Renal Biopsy Processing Standard Operating Procedure**. The following procedures **MUST** be followed for all specimens.

1. PURPOSE

To describe the procedure for processing and freezing renal biopsy tissue for assessment of gene expression in isolated immune cell subsets.

2. REQUIRED SUPPLIES

Items Supplied by ITN:

- CryoStor CS10 (BioLife Solutions, #210102)
- HypoThermosol (BioLife Solutions, #101102)
- 1.8mL cryovials
- Mr. Frosty Container (filled with isopropanol and pre-chilled at 4°C for at least 4 hours)

Items Supplied by Clinical Sites:

- 70% Ethanol
- Sterile forceps
- Sterile Serological Pipettes (1 mL)
- Ice bucket with wet ice
- Isopropanol
- Biopsy tray

Equipment Supplied by Clinical Sites:

The following laboratory equipment is required for renal tissue processing.

- Serological Pipettors
- BSL 1-2 Safety Cabinet
- -70 to -80°C Mechanical Freezer (preferably on back-up power and monitored for temperature)
- 4°C Refrigerator
- Flammable Safety Cabinet for Isopropanol Storage (preferred)

3. PROCEDURES

General Precautions

- Informed consent must be obtained from all participants before any research procedures, including specimen collection, are performed.
- Always follow appropriate precautions for handling of human specimens, including appropriate use of personal protective equipment.
- Perform all processing in a BSL 1-2 Safety Cabinet.
- Always use sterile technique.
- Always prepare materials and workspace by wiping with 70% ethanol.
- Before working with isopropanol, individuals should be trained in its proper handling and storage. For reference: <http://www.labmanager.com/lab-health-and-safety/2008/12/working-with-isopropyl-alcohol?fw1pk=2#.VsuyffkrLIU>.

Storage and Preparation of Reagents

- Store all reagents at 4°C, unless otherwise noted.
- HypoThermosol and CryoStor CS10 are cGMP-grade solutions. Use sterile technique when handling at all times. This includes minimizing exposure of solution to environmental contaminants.
- Be sure the Mr. Frosty container is filled with isopropanol and pre-chilled at 4°C for at least four hours before use. Note that the isopropanol in the Mr. Frosty should be replaced after every five uses, per manufacturer's recommendation.
- Isopropanol: The compound should be stored in a tightly closed container in a cool, dry, well-ventilated area away from incompatible substances. It should be kept away from heat, sparks, flames and other sources of ignition, as well as strong oxidizers, acetaldehyde, chlorine, ethylene oxide, acids, and isocyanates. Isopropyl alcohol is highly flammable and can easily ignite. Vapors may form explosive mixtures with air, traveling to a source of ignition and flash back. Use of water spray to fight fires may be inefficient. A flammable safety cabinet is the best storage option.

Tissue Handling Procedure

1. Wipe HypoThermosol container with 70% ethanol prior to use.

2. Label one sterile 1.8 mL cryovial with the Participant ID number using an alcohol-proof black pen.
3. Using a sterile 1 mL serological pipet, add 1 mL HypoThermosol to this 1.8 mL pre-labeled cryovial and place the cryovial on ice.
4. Using sterile forceps and sterile technique, add the renal biopsy specimen to the cryovial with HypoThermosol as soon as possible to avoid drying out and contamination.
5. Using a sterile 1 mL serological pipet, fill the cryovial with HypoThermosol to the brim so that the entire specimen is submerged and close the cap tightly.
6. Keep upright and on ice for 20-30 minutes before starting the cryopreservation procedure below. Do not freeze the renal tissue and do not leave at room temperature.

Tissue Cryopreservation Procedure

1. Wipe CryoStor CS10 container with 70% ethanol prior to use.
2. Using a sterile 1mL serological pipet, add 1 mL CryoStor CS10 to a pre-labeled 1.8 mL cryovial using sterile technique and place on ice.
3. Uncap the 1.8 mL cryovial containing the specimen in HypoThermosol. Using sterile forceps, transfer the specimen to the 1.8 mL cryovial containing CryoStor CS10.
4. Cap the 1.8 mL cryovial containing the specimen securely and keep it upright and on ice for 20 minutes. Do not freeze or leave at room temperature.
5. Place the cryovial containing the specimen in CryoStor CS10 in a Mr. Frosty container (filled with isopropanol and pre-chilled at 4°C for at least four hours).
6. Place the Mr. Frosty container in the -70 to -80°C freezer.
7. After 15 minutes, slap the Mr. Frosty container to facilitate nucleation, and return the Mr. Frosty to the -70 to -80°C freezer for a minimum of 24 hours and maximum of 7 days.
8. Transfer the cryovial from the Mr. Frosty to a storage box, and store at -70 to -80°C until shipment.