

Preservation in RNAlater®

A. Sample processing and storage

The objective is to preserve **both tumor tissue and normal adjacent tissue**. If possible the sample of tumor should be cut from the centre of the tumor, and the sample of normal tissue should be cut from a distant area of the tumor (i.e. Not marginal normal tissue)

Prepare the tissue samples on a clean surface and by using clean instruments (Change or clean instruments between preparing normal and tumor tissue). The ideal size of tissue is approximately 2.5cm x 2.5cm x 2.5 cm.

Preservation in RNAlater®

1. Making sure not to mix tumor and normal tissue samples, submerge each sample in one tube of 0.5ml RNAlater, which will have been pre-labeled. The material needs to be submerged in the solutions and weights no more than 500mg (maximum weight allowed in 1.8ml RNAlater tube for optimal preservation).
2. Place the tissue in the pre-labeled cryotube or small sterile plastic bags, making sure not to mix tumor and normal samples. Close the tubes or bags.
 1. Place the cryotubes or bags directly into the liquid nitrogen transport vessel.
 2. Transport to the storage location.
 3. Store the tube in a liquid/vapor nitrogen tank or in a -80⁰C freezer.

D. Sample storage

Store locally all obtained samples at -75 to -80⁰C. **Record the number and the type of stored samples, as well as the delays between tissue collection and preservation**, in the excel database. Enter storage location in your local tracking system, if available.

Snap Freezing

II. FRESH TISSUE SAMPLES (ORAL CAVITY, LARYNX and PHARYNX)

Whenever possible fresh tumor tissue will be collected. This will require a high level of coordination with surgeons.

A. Material used

1.8ml cryotubes (blue tops) or plastic bags

B. Sample collection

Fresh tissue specimen is collected at the time of biopsy or surgery. Specimen must be put in a sterile pot or bag and keep on ice. Specifically ask the surgeon NOT to immerse the specimen in formalin. **Strive to preserve all tissue samples within 30 minutes of excision from patient.** Tissue subject to delay of up to 2 hours should still be collected and the delay noted within the local inventory database.

C. Sample processing and storage

The objective is to preserve **both tumor tissue and normal adjacent tissue.** If possible the sample of tumor should be cut from the centre of the tumor, and the sample of normal tissue should be cut from a distant area of the tumor (i.e. not marginal normal tissue)

Prepare the tissue samples on a clean surface and by using clean instruments (Change or clean instruments between preparing normal and tumor tissue). The ideal size of tissue is approximately 2.5cm x 2.5cm x 2.5cm.

Cryopreservation (snap freezing)

1. Place the tissue in the pre-labeled cryotubes or small sterile plastic bags, making sure not to mix tumor and normal samples. Close the tubes or bags.
2. Place the cryotubes or bags directly into the liquid nitrogen transport vessel.
3. Transport to the storage location.
4. Store the tube in a liquid/vapor nitrogen tank or in a -80°C freezer.

Snap Freezing

D. Sample storage

Store locally all obtained samples at -75 to -80⁰C. **Record the number and the type of stored samples, as well as the delays between tissue collection and preservation,** in the excel database provided by IARC. Enter storage location in your local tracking system, if available.