

Tissue processing

Supplies

Liquid nitrogen in appropriate LN2 transport container

Isopentane stored at room temperature in a flammable cabinet

RNAlater

Tissue Tec OCT cryo-compound at room temperature

Tissue Cryomolds.

10% Neutral-Buffered Formalin

Saline

RPMI Medium 1640-used for the culture of human normal and neoplastic leukocytes,

PBS (Phosphate Buffered Saline )

Processing methods

1. Flash Freezing

Freezing by direct immersion in liquid nitrogen is common, although it is more damaging to the tissue. Rapid freezing in isopentane results less tissue damage.

Isopentane is an extremely volatile and flammable liquid at room temperature and pressure.

Vapor or mist is irritating to the eyes, skin, mucous membrane, and upper respiratory tract. Precaution should be taken to avoid contact to the skin and other body surface.

Pour approximately 100ml-200ml isopentane into a plastic beaker. Place the beaker into liquid nitrogen or dry ice. Isopentane can be cooled to its freezing point (-160°C).

The pre-labeled cassette with sample will be placed in isopentane cooled by dry ice or liquid nitrogen. Do not remove the tissue from isopentane until freezing is complete (10 seconds or less depending on size) but ensure sample does not crack.

Isopentane disposal method: allow gas to vent slowly to atmosphere in an unconfined area or exhaust hood.

## 2. Remain in RNA Later

As specific and non-specific RNA degradation begins immediately after tissue harvest, prompt transfer of fresh specimens into an RNA stabilization reagent is helpful to minimize changes in gene expression, and to ensure accurate quantitative analysis of gene expression.

RNAlater can be stored at room temperature. If any precipitation is seen, heat it to 37°C and agitate to redissolve. RNAlater can safely be discarded down the sink and flushed with water.

Tissue stored in RNAlater is initially kept overnight at 2°-8° C (minimum 12 hours).

After incubation in RNAlater at 4 ° C overnight, the following options are available until processing begins:

Samples can remain in RNAlater for up to 1 day at 37°C, 7 days at 18–25°C, or 4 weeks at 2–8°C, allowing processing, transportation, storage, and shipping of samples without liquid nitrogen or dry ice. Alternatively, the samples can also be placed at –20°C or –80°C for archival storage<sup>1</sup>.

## 3. OCT embedding

OCT protocol is used for freezing tissue samples when good preservation of histological details is required. OCT samples can also be used for protein, DNA and RNA extraction if there is enough tissue.

Put drops of OCT into a plastic cryomolds. Place tissue on top in correct orientation for cutting. Then carefully pour OCT on top of tissues to make sure none of the tissues remain exposed. Bubbles should be avoided.

Submerge cryomold with OCT embedded sample into isopentane cooled by dry ice or liquid nitrogen until OCT has hardened. OCT specimens will be subsequently placed either in -80°C freezer or in liquid nitrogen tank for storage.

## 4. Keeping tissue fresh for subsequent cell culture

Separate blades or scalpels or razor blades for bisecting samples should be used for each anatomic site to avoid cross-contamination.

Tissue sample should be placed into 15ml or 50 ml conical tube filled with at least 10 times of lab-made specific medium or the following reagents in a timely manner.

1. RPMI Medium 1640-used for the culture of human normal and neoplastic leukocytes,
2. PBS (Phosphate buffered saline )

Keep the tissue 4 °C until tissue distribution.

## 5. 10% Formalin fixation

10% Neutral-buffered formalin (10%NBF) contains 4% formaldehyde, it is the most common used fixative in pathology.

It is toxic by inhalation and if swallowed, can cause irritation to eyes, the respiratory system and the skin. Precaution should be taken to avoid inhalation, and contact the skin and other body surface.

Formalin should not be discarded down the drain. Refer to the chemical disposal plan for appropriate method of disposal.

### Tissue fixation in Formalin

Tissue should be no bigger than 20 x 10 x 5 mm.

The volume of formalin should be a minimum of 15 times the volume of the tissue samples. Containers should be capped, leak-proofed.

The advantage of formalin fixation is that tissue morphology is maximally preserved.

The most common molecular change induced by formalin fixation is the formation of cross-links between proteins, or between proteins and nucleic acids. This may be responsible for the masking the epitope by altering the three dimensional structure of the protein. Enzyme digestion or heat-induced epitope retrieval may rescue unavailable sites 2, 3.

### Duration and timing of formalin fixation

Tissue fixation time is dependent on tissue size. Small tissue piece (10 x 10 x 3mm) fixed in 10% NBF for 6 to 24 hours will generally show good cytological preservation.

#### 1. Short formaldehyde fixation before tissue dehydration and embedding in paraffin.

Short fixation results in cross linking only at the periphery of the tissue block. Coagulation by alcohol during tissue dehydration does occur in the center of the tissue, or the center actually remains raw and not fixed. Intensity of immunostaining of center area will be varied when comparing the well-fixed periphery area.

#### 2. Prolonged formaldehyde fixation (over than 48 hours)

Prolonged formaldehyde fixation results in excessive cross-linkage, lead to weak or absent staining, though increasing antigen retrieval step may counteract the effect of prolonged Formaldehyde fixation<sup>4</sup>.

#### 3. Delay of fixation

Delayed fixation results in irreversible weak or absent staining resulted from protein dephosphorylation or protein degradation<sup>5, 6</sup>.

Suggestion: If specimen cannot be fixed in a timely manner, refrigeration of the specimen may be helpful and is recommended.

#### References:

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3. Fox CH, Johnson FB, Whiting J, Roller PP. Formaldehyde fixation. *J Histochem Cytochem* 1985; 33(8):845-53.
4. Werner M, Chott A, Fabiano A, Battifora H. Effect of formalin tissue fixation and processing on immunohistochemistry. *Am J Surg Pathol* 2000; 24(7):1016-9.
5. Bai Y, Tolles J, Cheng H, et al. Quantitative assessment shows loss of antigenic epitopes as a function of pre-analytic variables. *Lab Invest* 91(8):1253-61.
6. Neumeister VM, Anagnostou V, Siddiqui S, et al. Quantitative assessment of effect of preanalytic cold ischemic time on protein expression in breast cancer tissues. *J Natl Cancer Inst* 104(23):1815-24.