

## Standard Processing Protocol for Fresh-Frozen (Snap-Frozen) Tissue

Surgically removed tissues must be frozen within 30 minutes after removal from the patient. Optimal sample size ranges from 0.3 cm to 0.5 cm.

## I. Using Dry Ice Freezing

1. After surgical removal, the excised tissue is placed in a styrofoam container with dry ice and embedded in the Optimal Cutting temperature (OCT) cryomold.

2. The OCT-embedded frozen sample is stored – 80°C.

## II. Using Liquid Nitrogen (LN2)

1. Place a cold insulating cylinder onto a stable and easy to access surface. Slowly pour liquid nitrogen (LN2) from the liquid nitrogen (LN2) storage container into the cold insulating cylinder. Fill to equal or less than half full.

2. Resected tissue sample should be placed in a labelled cryovial and immersed in the LN2 container using a pair of long tweezers.

3. Tissue is frozen within a minute and transferred at -80°C or to a vapor-phase liquid nitrogen storage tank.