Tips and recommendations for freezing specimens

The Early Access Program supports analysis of fresh-frozen tissue specimens using both the CODEX™ Antibody Kit 1.0 and custom CODEX™-tagged antibodies using the CODEX™ Conjugation Kit 1.0. While we have not screened all possible preparation protocols for generating tissue slices used in CODEX™ assays, this document serves to provide a few tips and recommendations to ensure you generate the highest quality data.

- All fresh-frozen tissues should be stored in OCT and sliced onto poly-lysine coverslips using a cryostat.
- Tissues that have been flash-frozen and stored in cryovials can be embedded into OCT prior to slicing. This process should involve minimal temperature changes to the tissue. The best way to ensure this, is to use a metal block immersed in liquid nitrogen. Pour OCT into the tissue mold and place on-top of the metal block, as the OCT is solidifying, but before it is fully solid, place the tissue in the OCT. Store tissue block in -80°C prior to slicing.
- For freshly collected tissues, minimize the time they are stored in room temperature. Store tissues in a PBS-based buffer prior to embedding in OCT. To embed tissues, fill a tissue mold with OCT, place tissue specimen in mold and flash freeze on top of a liquid nitrogen cooled metal block.
- Avoid multiple temperature changes once tissue block has been prepared.
- It is critical to quickly freeze tissue specimens in OCT to prevent artifacts at tissue borders.

3.3 Tissue Slicing

Fresh-frozen tissues for CODEX™ analysis should be sliced directly onto poly-lysine coated coverslips. Preparation and storage of tissue slices is critical for sample integrity.

Guidelines

Tissues

- Tissues sliced onto poly-lysine coated coverslips can be stored at -80°C for up to six months prior to staining.
- It is critical not to exceed 10 µm as this can disrupt the autofocusing capabilities of the microscope.
- For best quality CODEX™ data, tissue should be devoid of folds and tears.
- To ensure the integrity of the tissue slices it is critical that they do not stack on top of each other after placed on coverslips.

Tissue Placement on Coverslip

For Early Access, the tissues embedded on the coverslip and sample cassette are used together to form the sample well during the CODEX™ run. A 12mm x 15mm center portion of the coverslip will be the sample well. Figure 2 demonstrates the sample well that is created after the coverslip is mounted on the Cassette. Figure 3 illustrates the sample well size created with the coverslip and sample cassette. It is critical to center the tissue on the coverslip in what will be the well. If the tissue is not centered, it will not be in the well for the run. If the tissue exceeds past the edge of the sample well, a full seal will not be created and reagents will leak.

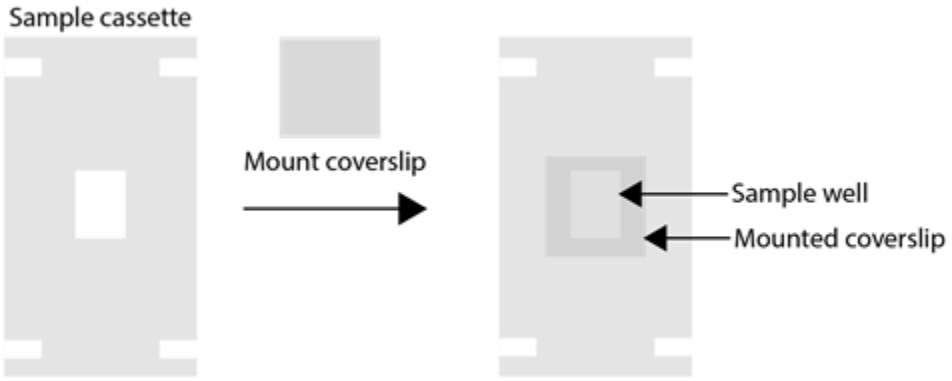


Figure 2: Each coverslip is 22mm^2 - it is mounted to the sample cassette to form a 12mm x 15mm sample well.

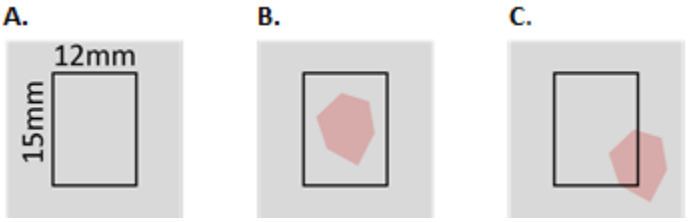


Figure 3: A) The sample well formed after mounting measures 12mm x 15mm. B) Tissues should be sliced onto the center of coverslips as shown in this schematic. This ensures the entirety of the tissue is within the sample well. C) This is an example of tissue placement on the coverslip that will result in improper mounting and run failure. This should therefore be avoided.

Pre-Experiment Preparation

Materials NOT included in kits:

- Poly-lysine coated coverslips prepared in section 3.1
- Cryo/Freezer box with tube inserts prepared in section 3.2
- Fresh/Frozen Tissue

Prepare Cryostat Chamber

Standard cryostats with temperature control are recommended for producing tissue slices. Most tissues will be sliced in temperature ranges from -15°C- -25°C. The exact temperature is unique to each tissue and is no different than what's typically used for standard slicing.

Slicing Tissue for Coverslip Adhesion

a. Set cryostats chamber to tissue specific range.
b. Place prepared tissue slicing storage box in cryostats chamber to equilibrate.
c. Place prepared poly-lysine coated coverslips in cryostats chamber to equilibrate.
d. Slice tissue between 5-10 µm.

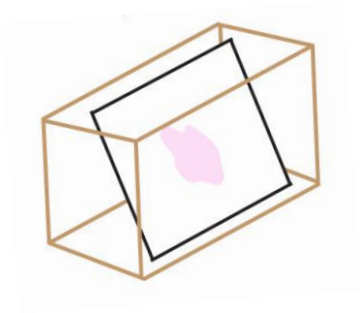
CRITICAL Do not to exceed 10 µm as this can disrupt the autofocusing capabilities of the microscope. Avoid folds and tears as this will effect proper data analysis.

e. Gently place tissue slice in center of coverslip such as Figure 3B
f. Adhere sliced tissue to the coverslip by placing a gloved finger on the underside of the coverslip just below the tissue for 1-2 seconds.

CRITICAL Do not keep your finger on the coverslip for more than the minimum time necessary to quickly melt OTC.

Note *This directed heat transfer should effectively melt the OCT and tissue, thereby ensure adherence. Chemical fixation will take place during the staining protocol.*

g. Place tissue slice in individual slot of prepared tissue slice storage box.



h. Repeat Steps d-g for each slice of tissue.
i. Once complete, cover tissue slice storage box with lid.
j. The box of tissue slices should be transported on dry ice to a -80°C freezer.

STOPPING POINT Samples can be stored at -80°C for up to six months prior to staining with care not to tip container.