

## INSTRUCTIONS FOR PREPARATION AND USE

- 1) Place the cryoEMBEDDER® and large, rectangular **button holder** inside the cryostat, allowing them to reach freezing temperature.
- 2) Insert the appropriate **adaptor** for your make of cryostat into the hole in the bottom portion of the cryoEMBEDDER®.
- 3) Place warm chucks in the **button holder** and immediately apply mounting medium.
- 4) Remove the desired number of **flat buttons** from the **cutting board** and place them in the freezing chamber of the cryostat for faster freezing.
- 5) Insert a **flat button** into the center hole in the top portion of the cryoEMBEDDER®. When the specimen is received and dyed, place it on the **flat button** with the cut side down and the skin side up.
- 6) Pick up the top portion of the cryoEMBEDDER® (with the **flat button** and the specimen) and begin teasing the edges and center of the specimen down, while holding it at eye level (can place on top of cryostat instead of holding in hand). The less time outside the chamber, the better.
- 7) Insert one of the prepared chucks into the hole between the pegs of the bottom portion of the cryoEMBEDDER®.
- 8) Apply mounting medium over the frozen tissue and *quickly* turn the top portion over onto the bottom portion, while holding the **flat button** in place where it overlaps the device.
- 9) The temperature of the cryostat chamber and the cryoEMBEDDER® will solidify the medium. However, spraying the medium with “cryo-freeze” will speed up the process.
- 10) Lift off the top portion of the cryoEMBEDDER® and remove the buttons that are stuck together with the frozen medium.
- 11) Slide the **flat button** off to the side by holding it by the stem on the bottom of the button. It is easier to remove when there is no medium around the edges of the button. If needed, insert the **razor blade** between the flat button and the medium to separate the two buttons.
- 12) Apply a small amount of medium over the exposed tissue and turn over onto the cold plate of the cryostat to create a buffer between the tissue and the knife blade. Trim off the excess medium and begin cutting or set aside and begin the embedding process with the next specimen.

### PURPOSE OF THE BUTTON HOLDER

The two-fold purpose of the **button holder** is to assist in the embedding of multiple tissue samples and to organize the specimens. It will retain its cold temperature for a considerable amount of time outside the chamber. Because there are small holes to accommodate the eight **flat buttons**, you can begin the freezing process on up to eight specimens at a time. After the specimens are properly oriented on the **flat buttons**, place the entire **button holder** with the eight **flat buttons** and specimens back into the freezing chamber and begin the next stage of embedding. The **button holder** is an organizer ideal for holding specimens both before and after cutting.

cryoEMBEDDER®

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