

**Procedure for submitting frozen sections - Only BSL-1 tissue NO BSL-2!!**

**Ordering information:**

Leica Biosystems Richmond  
P.O. Box 528  
Richmond, IL 60071  
800-225-3035  
FAX: 815-678-2216

**Tissue-Tek Accu-Edge Disposable Microtome Blades**

Cat. No. 25608-964 – low profile  
PK of 50 - \$108.23  
VWR  
800-932-5000

**Disposable Base Molds**

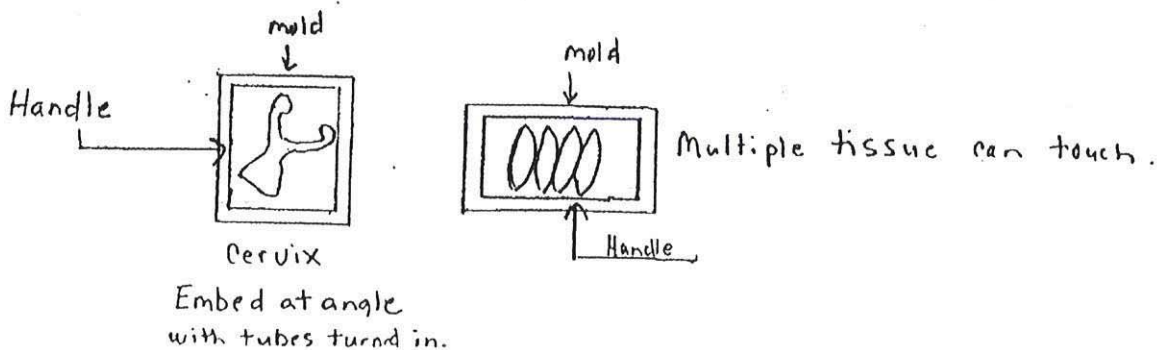
24X24 Cat. # 3803045 - \$43.34  
30X24 Cat. # 3803065 - \$43.34  
Both a box of 400

**FSC 22 Frozen Section Compound (Mounting Medium)**

Cat. No. 38014881 (Blue)  
Cs of 9 \$45.60

(FSC 22 is an embedding medium consisting of a mixture of polyvinyl alcohol and polyethylene glycol, which surrounds but does not infiltrate tissue. It freezes at -8°C).

1. Request form needs to be filled out with name, number to be reached at, fund/account number, PI's name, identification number for each slide block, slides to be cut from each block, sections/slide (usually 1-3 depending on tissue size), if the slides need to be fixed and what fixation to use and if staining required.
2. Tissue should be trimmed to the **thickness of a nickel** with **all fat removed around tissue** if not necessary **and fur shaved from skin**. Remember to label each cryomold with a tissue number. Place cryomold on dry ice (liquid nitrogen tends to make tissue hard and brittle) and fill it halfway with FSC 22 compound. Place tissue in cryomold and center/orient tissue(s) using bottom of cryomold as guide. Multiple tissues can touch. **It's important to have multiple tissues at the same level and direction. Do not let tissue touch sides of mold.** There needs to be a **'handle'** between the tissue and mold – see pictures below. Completely cover tissue to **top of mold** and freeze **quickly** on dry ice. **DO NOT** let FSC compound freeze and then add more. The block will split in half if not all frozen at the same time. The top surface needs to be smooth. Tissue will be popped out of mold and flipped to cut.
3. Blocks are routinely cut at 10µm unless otherwise requested.
4. Make sure to remove your slides/box of frozen tissue from Histology's freezer when notified they are cut. We have limited space and can not store your cut tissue.
5. When in doubt concerning proper procedures please call histology (262-1836) **before** continuing protocol.



**Recommended procedure for fixed tissue submission**

**Only BSL – 1 tissue NO BSL-2!!**

(This procedure does not affect immunohistochemistry protocols)

1. Dissect and fix tissue in fresh 4% paraformaldehyde on ice for 5-10 minutes.
2. Wash for 5 minutes in 1XPBS and repeat.
3. Transfer to 30% sucrose until the tissue sinks (5-10 minutes depending on the size of the tissue).
4. Transfer through a 1:1 mixture of OCT\*/sucrose and then into OCT\*.
5. Place the tissue in the cryomold, overlay with OCT\*, orient and freeze quickly on dry ice.
6. Once the tissue is in the mold with OCT\* it should be oriented and frozen quickly because a film can form on the top of the mold (where the OCT\* is exposed to air and make moving the tissue difficult).

\*Use OCT or FSC 22 embedding compound

0.2 M phosphate buffer

16.6 g  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$

2.5 g  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$

water to 400mL

4% paraformaldehyde (made fresh)

50mL water

4g. paraformaldehyde

heat to 60-70°C add 1 drop 10N NaOH and stir 10 minutes to dissolve add

50mL 0.2M phosphate buffer (final concentration 0.1M)

sterile filter and store on ice up to 1 day

30% sucrose in 0.1M phosphate buffer solution

100mL 0.2M phosphate buffer

60g. sucrose

dissolve and bring final volume up to 200mL with water