



PrestoCHILL New protocol

Application Note 03-2017

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NEW FEATURES

1. Fat tissues protocol

Fatty tissues are difficult to section using a cryostat because they are non-aqueous materials and simply do not freeze. The problem is clear when the fat begins to smear and impedes cutting of any tissue in its path. To make fat hard enough to section, it must be cooled to a very low temperature that often is too cold for non-fatty components present in the same sample. Another problem is that fat does not adhere well to the embedding medium.

Milestone studied a new protocol that allows fat cutting.

1. Prepare the mould by cleaning with paper soaked in alcohol and placing the appropriate chuck on it (this procedure will prevent ice inside the mould).

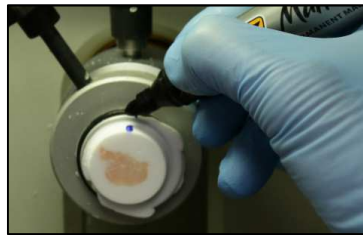


Cleaning of the mould is very important to avoid the block from sticking in the mould.

2. Prepare the sample on the spatula with thin layer of MCC medium under it. If preferable, it is possible to use the paper disk method (see BOP 102).
3. Transfer the sample inside the mould and immediately fill the mould with MCC medium.
4. Put chuck and heat extractor.
5. Freeze for 4 minutes at -40°C.
6. Trim the block in the cryostat and try to get a thin slice. It is possible that during the trimming the block temperature becomes too warm to section.



7. Put a mark on the block in order to recognize the position of the block inside the cryostat.



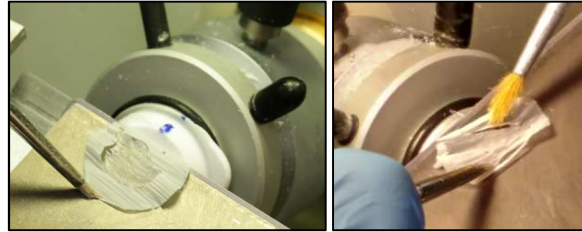
8. Cool down the block inside the PrestoCHILL for 1 minute. Be sure to put the block surface in contact with the PrestoCHILL cold plate: not in the mould used for the freezing because the thickness of the block now is reduced, it is better to use a larger mould or a free space on the metal plate.



9. After the 1 minute re-cooling, attach the chuck in its cryostat housing in the same position used for the trimming.



10. Cut immediately in order to get a slice before the warming up of the block. It is very important to cut the block quickly, moving the cryostat wheel very fast.
11. If, during cutting, the-section curls up, flatten it using two small brushes.



12. Pick up the section and fix immediately.

It is possible to repeat the cooling down of the trimmed block as needed to obtain a section

Milestone suggests the use of MCC medium for better adhesion between the sample and embedding medium. Thanks to its higher fluidity, MCC flows faster to surround and adhere to the samples.

If some non-fatty parts are present in the sample and they shatter, it is possible to warm these parts up with gloved thumb.

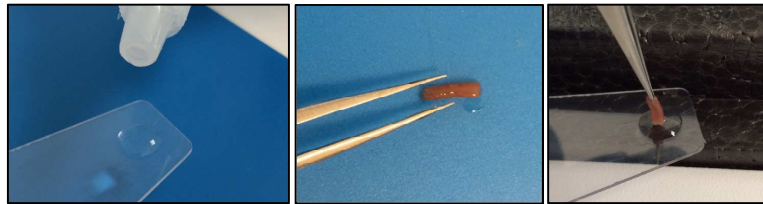
IMPROVED FEATURES

1. Muscle tissue protocol

Muscle tissues are very rich in water and this causes the formation, during freezing, of large ice crystals within and between the muscle fibres. In addition, it is important, in order to morphologically evaluate the muscle tissues, to have a cross section.

The PrestoCHILL is used to freeze the muscle specimen quickly and in the correct position, through a technique different from the standard one. Instead to put the specimen on the spatula and move it inside the mould through a sliding movement, the sample must be put directly into the mould.

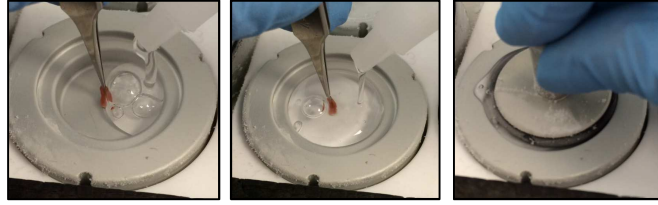
1. Prepare a drop of MCC on the tip of the spatula. Take the specimen with tweezers so as to have the section of interest free and wet it with the drop of MCC.



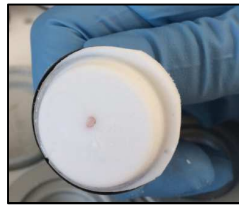
2. Place the specimen in the mould so that the muscle fibres are vertical, this will provide cross section orientation for the cut.



3. Holding the specimen firmly, fill the mould with MCC. Then place the chuck and heat extractor and wait for the freezing time to elapse.



4. Extract the block, cut and stain the slide.



At the end of the freezing and cutting processes, it is possible to follow the BOP 105 procedure in order to further improve the quality of the muscle tissue sections.



Mould with smaller well give a faster freezing and consequently a better quality. See code MLD4415 and MLD4410.

