calaCLEAR™

The high-resolution microscope slide

Instructions for use of the calaCLEAR microscope slide

For Paraffin Sections:

1) The calaCLEAR slide should always be handled so as to avoid touching the glass coverslip/window.

2) The calaCLEAR slide can be directly marked with specimen details using a pencil, permanent marker pen (e.g. microscope slide marker pen) or a UV-cure inkjet slide printer. Alternatively, a Calamat transparent polyester, xylene-resistant label can be applied and then marked by a slide printer. Extra protection of the slide identification can be achieved by using the Calamat transparent polyester, xylene resistant label as a protective "over-label".

3) Cut paraffin tissue sections on a microtome at between 2 and 5um and float out on the water bath (according to your usual practice).

4) Unlike when using a conventional glass microscope slide, with the calaCLEAR microscope slide the section is picked up onto the underside of the slide, **i.e. the side of the glass window that forms the base of the depression/well.**

5) Place the calaCLEAR microscope slide in a slide rack and heat in an oven for 20 minutes at 62 degrees centigrade (according to your usual practice).

6) De-wax, hydrate, stain and dehydrate/clear to xylene (or xylene substitute) in the normal way. The slide can be run on automated histochemical stainers.

7) For the calaCLEAR slide no coverslip need be applied; it is only necessary to seal the section onto the glass window. To do this use the calaSEAL sealant supplied. Add 400ul of prepared CALASEAL into the well and directly onto the stained tissue section and transfer to the 62 degrees centigrade oven. The solvent evaporates quickly and the slide is sufficiently dry for initial examination in less than one minute. In the 62 degrees oven, the sealant is fully dry in 10 minutes. On the bench at room temperature, the slide is fully dry within 30 minutes; faster in a fan-assisted drying chamber.

8) Once the sealant is sufficiently dried, examine the calaCLEAR slide on the microscope with the coverslip uppermost, i.e. the dried sealant is face down. The increased resolution seen with the calaCLEAR slide is most evident when viewed through objectives with high numerical aperture – i.e. x20 objectives with NA of 0.75 and higher.

For Frozen Sections:

When picking up a frozen section with a conventional glass microscope slide it is usual practice to touch the freshly cut frozen section with the slide. This "melts" the cryomountant that contains the section onto the glass. When using the calaCLEAR slide for frozen sections, a neat trick is employed. The freshly cut section cannot be picked up in the same way as when using a conventional glass slide because the under-surface of the glass window is 1mm from the frozen section (the thickness of the metal slide). Instead, the calaCLEAR slide window is placed over the frozen section and, wearing rubber or nitrile gloves, the operator gently rubs his/her finger on the topside of the glass window Static electricity is thus generated and the frozen section jumps onto the under-surface of the glass window where the cryomountant melts. This triboelectric effect permits accurate positioning of the frozen section in the very centre of the window. Furthermore, tests have shown that this method of attaching the frozen section results in greater adherence of the frozen section to the glass.
Once the frozen section is "picked-up" in this way, the frozen section is stained in the usual way and sealed with either aqueous mountant or, following clearing through to xylene (or xylene substitute), is sealed with calaCLEAR as described above for paraffin sections (steps 7 & 8). We recommend the use of calaSEAL after clearing and with rapid drying at 65 degrees for intraoperative frozen sections.

For Cytospin cytological preparations:

A cytospin preparation can be made on the calaCLEAR slide in the same way as for a conventional slide but with one modification - the cardboard filters are cut down in size to fit snugly into the metal hole overlying the glass coverslip window. Ensure that the hole in the cardboard filter remains aligned with the hole in the mounted cytospin assembly through which cells are delivered from the well to the glass coverslip window.

Preparation and application of CALASEAL sealant

For histology:

calaSEAL is prepared by dissolving two plastic pellets for each 1ml of final solution. The solvent is 50% acetone and 50% xylene (or xylene substitute). The calaSEAL solution can be stored in a stoppered bottle at room temperature prior to use.
The amount of prepared calaSEAL used to seal a single slide is 0.4ml (400ul). The prepared calaSEAL is dispensed with a standard laboratory pipette or repeater pipette. The solution is aimed at the centre of the tissue section and will flash spread to fill the base surface of the glass window. Accelerated drying (particularly for frozen sections) is achieved by placing the calaCLEAR slides (with the exposed sealant solution uppermost) on a rack in a laboratory oven set at 65 degrees centigrade.

For cytospin preparations:

The calaSEAL solution is prepared by dissolving two of the plastic pellets for each 1ml of final solution. The solvent is 50% acetone and 50% xylene (or xylene substitute). The solution can be stored in a stoppered bottle at room temperature prior to use.
A few drops of prepared calaSEAL sealant are dispensed with a standard laboratory pipette or repeater pipette. Drops of the solution are aimed at the centre of the cytospin preparation and will flash spread to seal the cells to the surface of the glass window.

If early inspection of the prepared slide is necessary, accelerated drying is achieved by placing the slides (with the exposed sealant solution uppermost) on a rack in a laboratory oven set at approximately 65 degrees centigrade.