

## Protocol

# HoloLid™ 71131

### STERILIZING

1. Place HoloLid into a cleansing bath with warm tap water and detergent for at least 10 minutes.
2. Rinse in multiple steps with tap water first and ultra-pure water last.
3. Place HoloLid into a bath with 70 % non-denatured ethanol inside the sterile bench for 5-15 minutes. It is important to keep the ethanol bath as short as possible, as ethanol affects the optical quality of the plastic. Keep HoloLid sterile and handle with sterile tweezers.
4. Let the HoloLids air dry inside the LAF-bench. Store in a sterile fashion until used. A square Petri dish of 100 × 100 mm is recommended.
5. After usage: Put the lids into a warm bath with tap water and detergent for approx an hour. Rinse thoroughly with tap water and continue with distilled water. Let them air dry until usage.



*Let HoloLid air dry in a tilted position.  
Keep HoloLid in a sterile environment.  
Never use paper or fabric; fibers will stick  
onto the surface and affect the image  
quality.*

### USAGE

**All steps below are to be handled with standard sterile procedures.**

1. Seed the cells. A working volume of 2.5 ml for each well is recommended. The volume is adjusted to reach a surface level that allows the observation window to be immersed. Remember to take into account that the volume of the treatment adds to the final working volume.
2. Put on the standard lid.
3. Let the cells adhere in the incubator for 1-24 hours, depending on the required adherence time for the specific cells used. This step is performed to avoid uneven distribution of cells. If a reagent is to be added one day after seeding, it is recommended to change lids after the addition. The plate is asymmetric. **Always place the plate with the cut corners to the left in all steps, from seeding, adding treatment, and mounting on the plate holder.**
4. Replace the standard lid with HoloLid. Make sure there are no air bubbles in the cell media before changing the lids. If there is an air bubble, it can be removed by blowing a little puff of clean air onto to bubble, which will burst. Clean air can be created by using an ethanol dispensing bottle with a little ethanol inside and the inner tube removed. Press the bottle carefully while targeting the bubble with its tip.
5. Sample is ready to be used.



*HoloLid placed on a ibidi  $\mu$ -Plate 24 well. The red circles shows the cut corners (left). The areas marked blue are the observation windows which are immersed into the cell media. The red single circle shows the geometric feature of HoloLid 71131, distinguishing from other similar HoloLids (right).*

### PRODUCT DESCRIPTION

HoloLid has been especially designed for the HoloMonitor® time-lapse cytometer to eliminate image disturbances caused by surface vibrations and condensation inside the cell culture vessel. HoloLid (cat. # 71131) is designed to fit ibidi cat. # 80241 for wound healing experiments in a 24 well format, but also fits ibidi  $\mu$ -Plate 24 Well (cat. # 82406) without culture inserts for wound healing. HoloLid can be reused at least 10 times, but please note that after extensive use, the repeated sterilization will noticeably degrade the optical quality of the lid.

### MATERIAL

HoloLid is made of poly methyl methacrylate (PMMA or Plexiglas). PMMA is a non-toxic material often used in medical surgery implants, dentures etc. It does not contain Bisphenol-A; a cell disturbing agent commonly present in plastics.

HoloLid is shipped with a **plastic cover that must be peeled off before use**. It is recommended to sterilize HoloLid before use.

### FURTHER INFORMATION

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