HOLOLID™ PROTOCOL
STERILIZATION AND USE

HoloLid™ has been specifically designed for the HoloMonitor® cell culture microscope to eliminate image disturbances caused by surface vibrations and condensation inside the cell culture vessel. The HoloLids should be used together with a compatible cell culture vessel to ensure optimal image quality. HoloLids can be used to image cells using other microscopes, such as confocal and fluorescense microscopes.

REQUIREMENTS:

- Detergent, such as dish soap
- Warm tap water
- 70 % Ethanol (v/v)
- Sterile LAF bench
- HoloLid™ for selected vessel
- Ultra pure water
- Tweezers
- Containers for liquids

Sterilization

1. **Place** HoloLid into a cleansing bath with warm tap water and detergent for **10-60 minutes**.
2. **Rinse** the detergent in multiple steps with tap water first and ultra-pure water last.
3. Place HoloLid into a bath with **70 % (v/v) 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.
4. Let the HoloLids **air dry** inside the LAF bench.
   ► **Store** in a **sterile** fashion until used and handle with sterile tweezers. A square Petri dish of 100 × 100 mm is recommended.

Before the first use: HoloLid™ is shipped with a plastic cover that must be peeled off before use. HoloLid™ should be sterilized before its first use.

Let HoloLid™ air dry in a tilted position.

Never use paper or fabric to remove liquid drops - fibers will stick onto the surface and affect the image quality.

Use

1. **Seed** the cells, put on the standard lid and **let the cells adhere** in the incubator for 1-24 hours (depending on the required adherence time for the specific cells used).
   ► **Always place the plate with the cut corners according to the table below** in all steps, from seeding, adding treatment, and mounting on the plate holder.

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.

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.

Scheelevägen 22, 22363 Lund, Sweden
+46 46 38 60 80 | support@phiab.se | www.phiab.com

2. **Replace** the standard lid with **HoloLid™**. **Make sure** there are **no air bubbles** in the cell media before changing the lids.
   ► Clean air can be created by using an ethanol dispensing bottle with inner tube removed, and which contains a small volume (100-150 mL) of 70% ethanol. Press the bottle carefully while directing the air stream towards the bubble. Do not touch the cell media.
3. **Sample is ready** to be used.

If there is an air bubble, it can be removed by blowing a little puff of clean air onto to bubble, which will burst.

HoloLids™ are compatible with these vessel formats


35 mm Petri dish


6-well plate


24-well plate

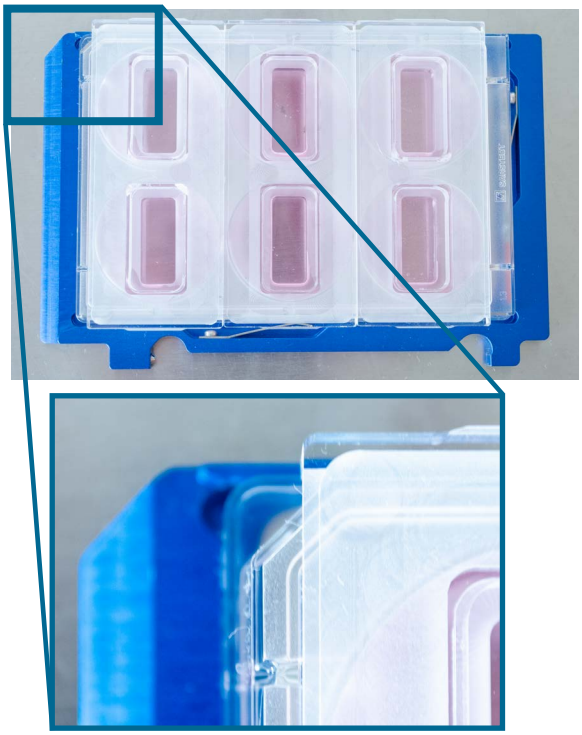

96-well plate

Vessel	Vendor cat. number	HoloLid™	Final working volume	Growth area, cm²/well	Cut corner orientation in a holder
Sarstedt TC-dish 35	83.3900	71110	3.0 mL/well	8.00	NA
Sarstedt TC 6-well plate	83.3920.005	71120	3.0 mL/well	8.80	top left
Sarstedt lumox® 24-multiwell plate	94.6000.014	71130	1.9 mL/well	1.90	top left
Sarstedt lumox® 96-multiwell plate	94.6000.024	71140	170 µL/well	0.34	top left
ibidi® µ-dish 35 mm, high	81156	71111	2.5 mL/well	3.50	NA
ibidi® µ-plate 24 Well Black	81156	71131	2.5 mL/well	1.90	NA
Eppendorf CCCadvanced® FN1 - 6 well	0038110010	71150	3.0 mL/well	9.40	bottom right

Fitting cell culturing plate to vessel holder

1. Wipe the **Vessel holder** with ethanol and put it in the LAF bench, the grips facing towards the user.
2. Place the cell sample onto the HoloMonitor® M4 **Vessel holder** by letting it slide on the track on the bottom. Ensure that the vessel is parallel to the holder. There is a spring to hold the vessel in place.
   ► Orient the plates' cut corner according to the table above.

HoloLids can be purchased from phiab.com or via your local distributor.


Example of the vessel cut corner orientation in a vessel holder