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Figure 1. H201-NIKON-TI-SR - Components and Dimensions.

2. Sample Holders

2.1 Available Sample Holders

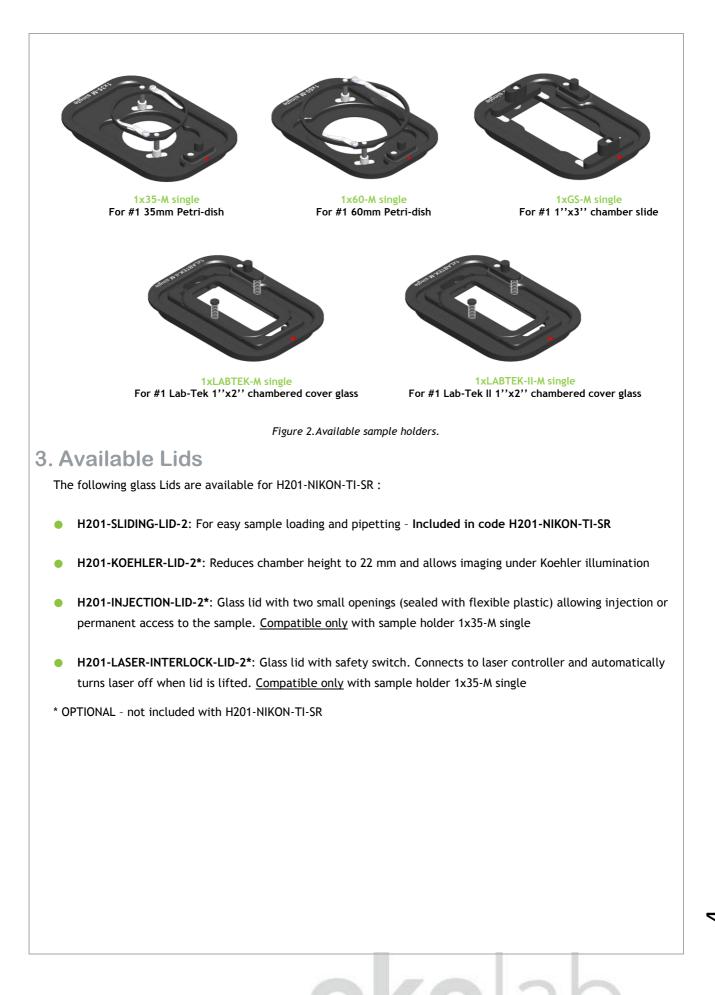
The following sample holders are available.

NOTE: Please contact <u>info@oko-lab.com</u> if you cannot find the sample holder you are looking for. We are constantly adding new inserts to the list.

1x35-M single	#1 35mm Petri-dish
1xGS-M single	#1 1x3in. chamber slide
1xLABTEK-M single	#1 Lab-Tek 1in.x2in. chambered cover glass
1xLABTEK-II-M single	#1 Lab-Tek II 1in.x2in. chambered cover glass
1x60-M single	#1 60mm Petri-dish

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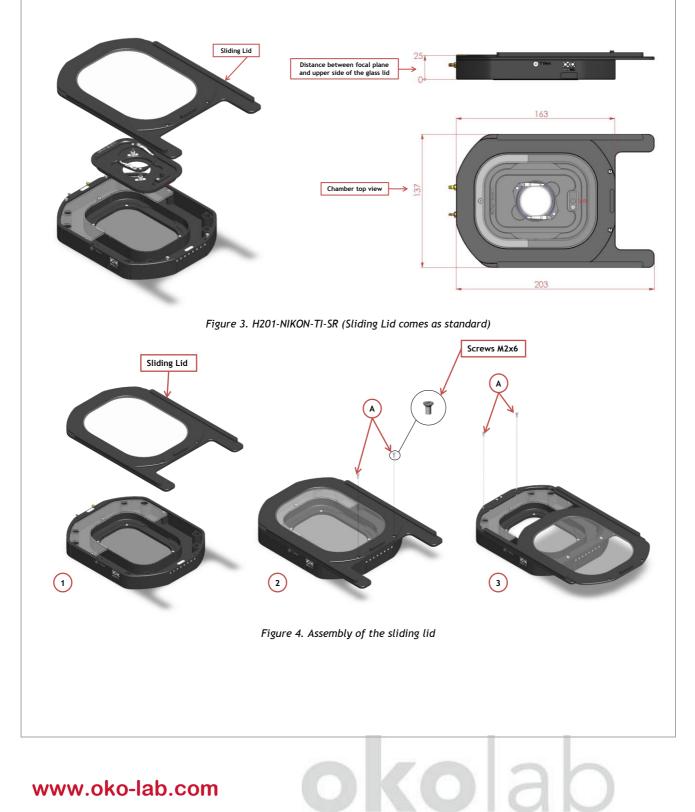
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3.1 Sliding lid

The Sliding Lid is a glass lid allowing for easy sample loading and pipetting. The Sliding Lid is screwed onto the chamber.

Figure 3 shows chamber dimensions with the Sliding Lid. Figure 4 illustrates how to remove Sliding Lid, when a different Lid is necessary.

NOTE: Sliding Lid MUST BE REMOVED when using any other Lid. The Sliding Lid is fixed onto the chamber with 4 screws. Screws location is indicated by letter A in Figure 4 (image 2 and 3). Keep Sliding Lid fully closed to access screws labeled A in image 2. Open Sliding Lid to access screws labeled A in image 3.



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3.2 Koehler Lid

The Koehler Lid is a glass lid reducing the chamber's height to 22 mm and allowing imaging under Koehler illumination. The Koehler Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

Figure 5 shows chamber dimensions with Koehler lid.

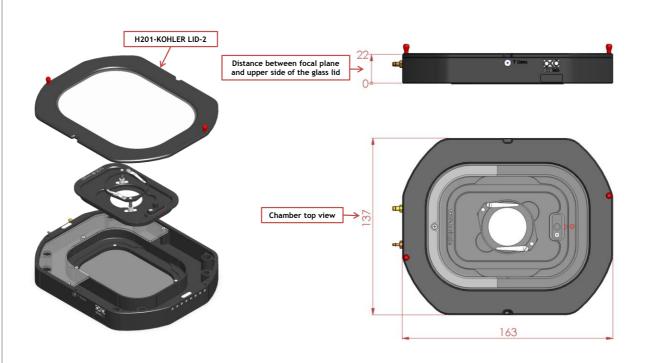


Figure 5. H201-NIKON-TI-SR + H201-KOEHLER-LID-2

3.3 Injection Lid

The Injection Lid is a glass lid with two small openings sealed with flexible plastic allowing injection or permanent access to the sample. NOTE: Compatible with sample holder 1x35-M single ONLY. The Injection Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

Figure 6 shows chamber dimensions with the Injection Lid.



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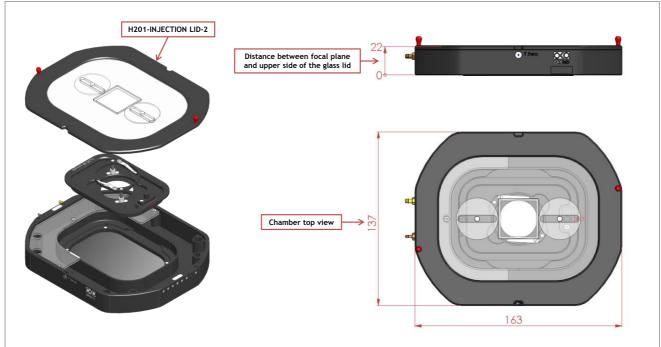
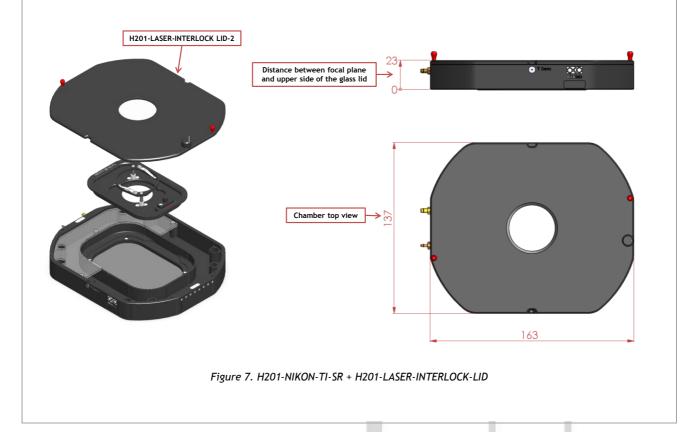


Figure 6. H201-NIKON-TI-SR + H201-INJECTION-LID

3.4 Laser Interlock Lid

The Laser Interlock Lid is a glass lid with a safety switch connecting to the laser controller. It automatically turns the laser off when the lid is lifted. NOTE: <u>Compatible with sample holder 1x35-M single ONLY</u>. The Laser Interlock Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

Figure 7 shows chamber dimensions with the Laser Interlock Lid.





4. Insertion of the Sample Feedback Temperature Sensor

Insert the Sample Feedback Temperature Sensor through the dedicated opening located in the H201-NIKON-TI-SR (see Figure 8, Frontal and 3D views).

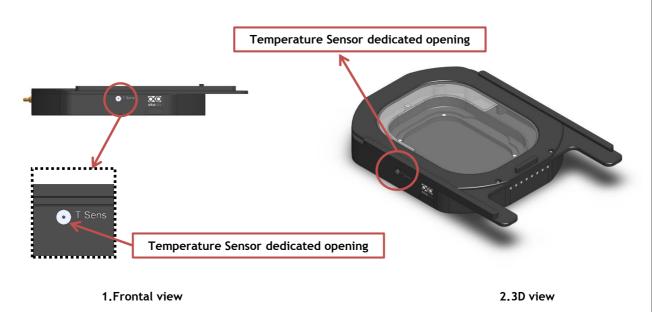


Figure 8. Insertion of the temperature sensor inside the chamber.

5. Insertion of Sample Holder into Chamber

Sample holders fit into the chamber base and are held in place by magnets embedded within both chamber and holder. To introduce a sample holder with the proper orientation, match the red dot on the holder to the one on the chamber base, as illustrated in Figure 9.

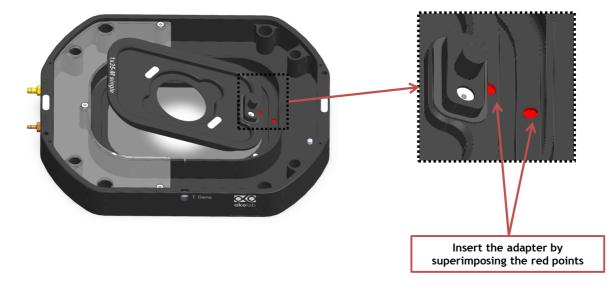


Figure 9. Introduction of the Specimen Holder inside the Chamber Base.



6. Working with 35 or 60 mm Petri Dish – Spacing Rings and Magnetic Locks

Magnetic locks prevent movement of 35 and 60 mm dishes inside the sample holder.

Figure 10 illustrates the available magnetic locks for 35 and 60 mm dishes. Threaded magnetic posts allow adjusting holder's height.

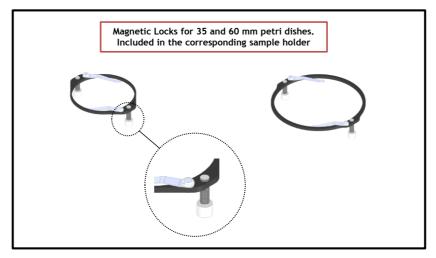


Figure 10. Magnetic locks for 35 and 60 mm dish

NOTE: Magnetic locks are included with sample holder.

Spacing rings with variable diameter are also included in according to the needs of the user. Figure 11 shows how to position the ring inside the sample holder. Ring selection guide diagrams: rings for 35 and 60 mm dish -Figure 12 and Figure 13, respectively.

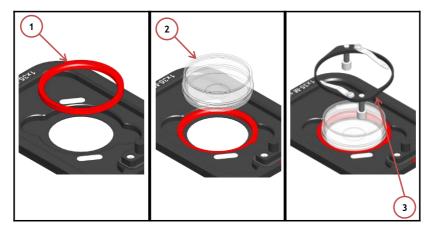
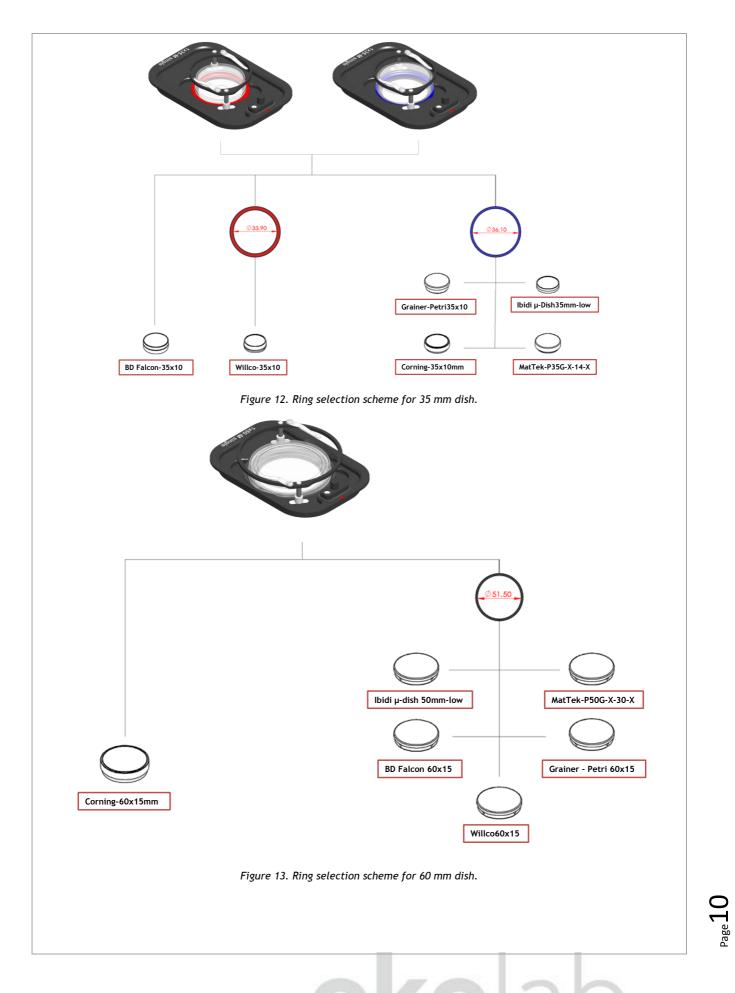


Figure 11. 1) Insertion of the ring, 2) insertion of the 35 mm dish, 3) insertion of the magnetic lock. **NOTE:** Spacing rings are included with sample holder.







7. Working with 1x3" and 1x2" chamber slides - magnetic locks

Magnetic locks prevent movement of 1''x 3'' and 1''x 2'' chamber slides inside of the sample holder.

NOTE: Magnetic locks are included with sample holder.

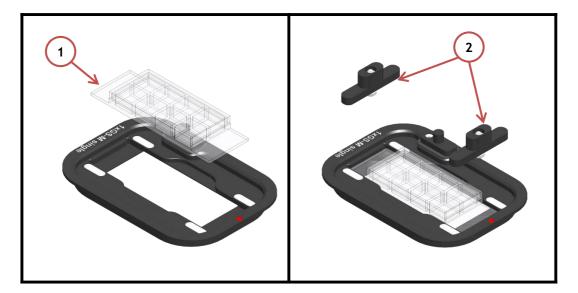


Figure 14. Magnetic lock for 1" x 3" chamber slide.

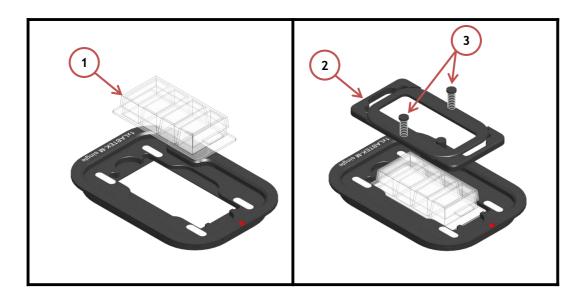


Figure 15. Magnetic lock for 1" x 2" chamber slide. To lock the slide, push simultaneously the buttons indicated with (3).

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8. Connecting the Gas Supply and Filling the Humidity Module

Single silicon tubing carries output gas from the Okolab Gas Controller to the H201-NIKON-TI-SR. Silicon tubing connects to a gas input - brass opening - located on a side of the H201-NIKON-TI-SR. See Figure 16. Connect the silicon tubing by gently pushing it onto brass opening.

H201-NIKON-TI-SR includes an integrated humidity module in order to maintain a high humidity percentage inside the chamber. Fill the humidity module inside the chamber through the water input - brass opening - located on a side of the H201-NIKON-TI-SR (See Figure 16) using a silicon tubing 2 mm ID and a syringe*.

*NOTE: Do not exceed 0.09 l



Figure 16. Connection with gas supply.

9. Working with Perfusion

H201-NIKON-TI-SR features 8 perfusion holes for the insertion of perfusion tubing up to 2.5 mm in outer diameter. Small screws plug the perfusion holes when not in use. (Grub screws M3x3). <u>Remove small screws</u> as needed before introducing perfusion tubing.

Figure 17 shows location of perfusion holes.



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10. Connecting the Chamber with XY stage

Follow the steps shown in the images Figure 18 and listed below in order to correctly connect the chamber with XY stage.

- 1. Place the chamber on the stage and tighten 4 captive screws (See Figure 18 Image 1 and Image 2). Captive screws housings are indicated with letter B in Figure 18 Image 2
- Use a 2.5mm metric Allen Wrench to tighten the four captive screws while keeping the chamber sliding lid open (See Figure 18 Image 2)

