

Tokai Hit Stage Top Incubator: Instructions for Use

Model: INUB-GSI-BP-F1

Start-up and Use:

1. Turn on the microscope according to the posted Startup protocol.
2. Move the objective to its lowest point using the focus knob or the “down arrow” on the right of the microscope for determining boundaries.
3. Install the stage plate of the chamber unit on the stage and secure with the fixing screws.
4. If using perfusion pipes (for experiments with long periods without a dish lid – can cause problems with culture media evaporation), remove the “shoulder screws” at both ends of the chamber unit, place the pipes in the perfusion grooves and hold loosely with the shoulder screws. Place the dish inside the chamber unit, adjust the pipes position and screw the shoulder screws tightly to fix the pipes in place.
5. Check that the Water Supply Tube is clipped shut. Fill the internal water bath (around the periphery) ~ 3/4 full with distilled water (up to the Gas Supply Tube level). Add the water in multiple places for good distribution. Be very careful not to overfill!
6. Put an empty dish in the dish attachment to cover the opening. These are available in the box on the shelf with the stage inserts. **Warning!** If device is operated without a dish set in the Chamber Unit, condensation may build up on the objective.
7. Lower the dish holder into the chamber unit, pushing down gently on all corners to secure it into place.
8. Install the top heater, checking all corners to ensure that it is seated properly. Wrap objective with lens heater.
9. If using CO₂, open the main valve on the CO₂ tank, open the secondary valve (turn the small black knob on the left to go up) and gently turn the large black knob to adjust the pressure on the left-hand gauge to approximately 15 PSI. **Note: we do not have CO₂ installed yet.**
10. Press the main power button on the controller. Then, turn on the switches for the
 - Top Heater
 - Bath Heater

- Stage Heater
- Lens Heater
- CO2 Concentration ***Don't turn on until we have the CO2 tank set up.***

11. Wait 30 minutes for the incubator to equilibrate.
12. When you are ready to image, replace the empty dish with your sample.
13. For multi-day time-lapse experiments, replenish the water bath as necessary (to do this, carefully supplement with a syringe through the Water Supply Tube connection, approximately every 12 hours).

Shutdown

1. On the controller, turn off all component switches then turn off the main power.
2. On the CO2 tank, close the secondary CO2 valve, then close the main valve.
3. Use a transfer pipette to remove as much residual water from the internal water bath as possible. Use cotton swabs or Kimwipes to remove any remaining water. Wipe down with EtOH to prevent mold growth.
4. Remove Lens Heater from objective.
5. Remove the entire stage top unit from the stage and put carefully back in the box on the shelf.
6. Shut down the microscope system as posted and clean the objective.