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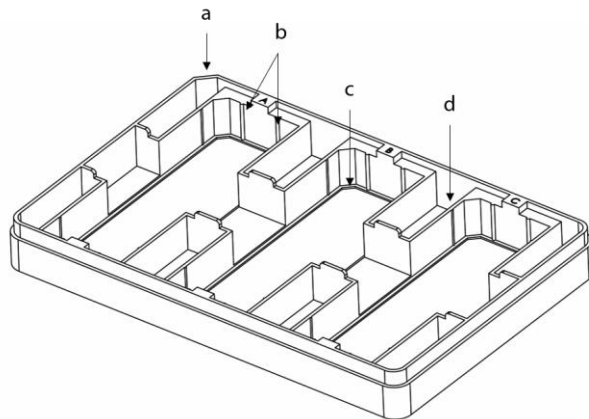
## INTRODUCTION

HOL-2 Chip Holder is a sterile, single use, stackable holder that can fit up to three AIM Chips in three slots. It features 8 reservoirs for humidity control and 4 bumps in each slot to secure the chip. This protocol covers the assembly and removal of the AIM Chips onto/from the AIM Holder as well as the humidity control measures within the AIM Holder.

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## SCHEMATIC

The following schematic shows the 3D presentation of the AIM Holder. This nomenclature will be used extensively in this protocol.



Nomenclature:

- a : chamfer
- b : bumps
- c : ledge
- d : reservoir

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## AIM CHIP ASSEMBLY TIMING 2 min

### MATERIALS.

- AIM Holder
- AIM 3D Cell Culture Chips

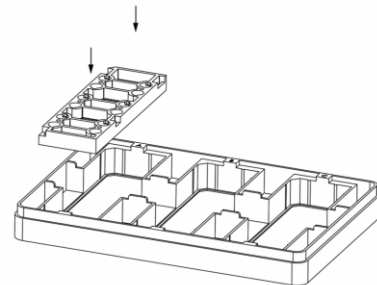
### Others

- 37°C incubator

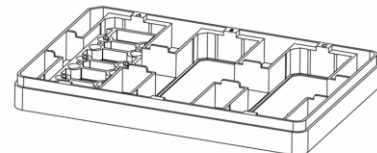
1. Align the AIM Chip to the AIM Holder by matching their chamfers.
2. Push the AIM Chip downward until it is flush against the ledge. The tightening bumps provide sufficient resistance to hold the chip in place.

**! Critical** Avoid touching the media ports and gel inlets when pushing the AIM Chip downward.

(I) Align and push the chip firmly into the slot



(II) Assembled chip



**Reminder** Ensure that the Chip is flush against the ledge for consistent culturing and imaging conditions

## AIM CHIP REMOVAL TIMING 2 min

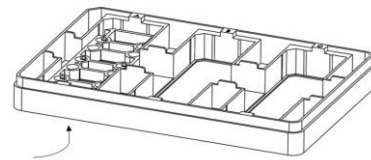
### MATERIALS

- AIM Holder with assembled AIM Chips

3. Remove water from all reservoirs (if any) and medium from all ports of the AIM Chip that is going to be removed from the holder.
4. Hold the AIM Holder on the long sides with both hands.
5. Place your index fingers and thumbs on the bottom and the top of the AIM Chip, respectively.
6. Exert a gentle upward force with the index fingers to lift the AIM Chip. Once the AIM Chip is sufficiently lifted, it can be removed by holding onto the long side of the chip.

**Reminder** Do not empty the media in the channels.

**Reminder** Repetitive assembly and removal of chips may smoothen the bumps thus reducing the grip strength on the chip.



Gently push the AIM Chip up

**! Critical** Abrupt force exertion on the AIM chip may cause it to flip thus increasing the risk of cross-contamination.

## HUMIDITY CONTROL TIMING 5 min

Optional: Humidity control is needed if the evaporation of medium cannot be tolerated. The AIM Holder houses reservoirs to create a humidified environment to limit evaporation from the AIM Chip to as low as 1% over 24 hours when kept in a CO<sub>2</sub> incubator.

There are 8 inbuilt reservoirs on the AIM holder: 4 smaller reservoirs on the side of the Holder and 4 larger reservoirs interspersed between the Chip slots.

### MATERIALS

#### Reagents

- Sterilized water

#### Others

- AIM Holders with assembled AIM Chips

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7. Add 2 ml of sterilized water into each of the larger reservoirs and 1 ml of sterilized water into each of the smaller reservoirs.

**Reminder** Handle holders with care as the water in the reservoirs may spill. Other alternatives for humidity control such as low percentage agarose gel or wetted sterilized paper towel may also be used if spillage of water is a concern.

8. Cover AIM holders with the dedicated covers.

9. Replenish the water in the reservoirs weekly or when the water cannot cover the full surface of the reservoirs, whichever comes first, for long term cell culture.

**Reminder** The evaporation rate also depends on the position of AIM holders in an incubator, the model of the incubator and the frequency of the incubator being accessed.