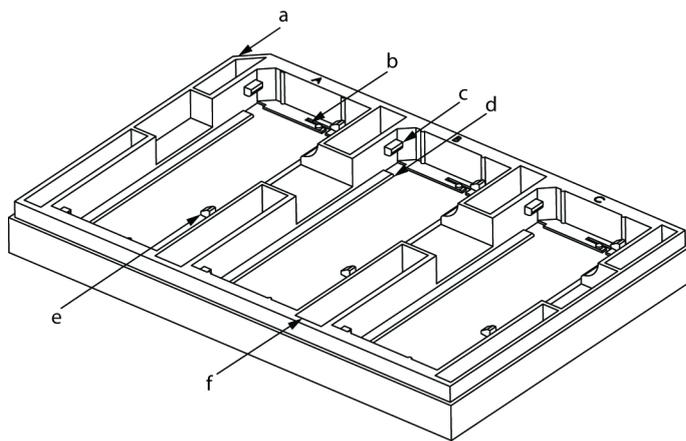


## INTRODUCTION

HOL-1 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; two snaps and two catches in each slot for holding the AIM chip in place; two lifters for easier chip removal. This protocol covers the assembly and removal of the AIM chip onto/from the chip holder as well as the humidity control measures in the chip holder.

## SCHEMATIC

The following schematic shows the 3D presentation of a chip holder. This nomenclature will be used extensively in this protocol.



Nomenclature:

- a : chamfer
- b : lifter
- c : catch
- d : ledge
- e : snap
- f : reservoir

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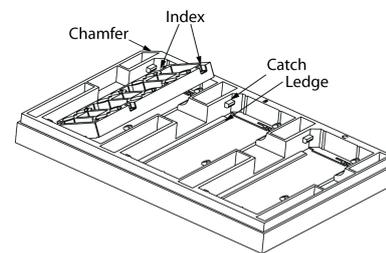
## AIM CHIP ASSEMBLY TIMING 2 min

### MATERIALS.

#### Others

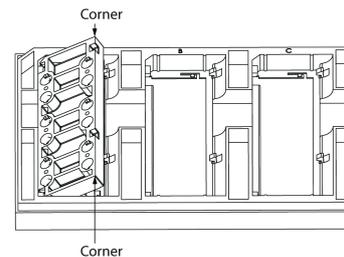
- AIM holder
- AIM 3D Cell Culture Chips

1. Align AIM chips to the chip holder by matching their chamfers.
2. Tilt AIM chips and slide them into the space between the catches and the ledge and make sure the catches and indexes are matched.

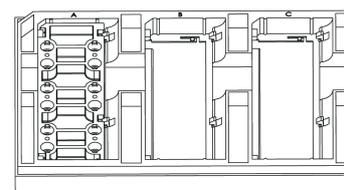


3. Press down the two corners of AIM chips (the side in contact with snaps) until the AIM chips sit in tightly. The snaps will hold the chip in place.

(i) Press these two corners



(ii) The AIM chip is held in place by the snaps and catches



**! Caution** Snaps may break and cause impact hazards if the holder is handled inappropriately. Always perform chip assembly in a laminar flow hood with the window sash lowered to the appropriate level. Put on safety spectacles with side shields if the assembly has to be done outside a laminar flow hood.

**! Critical** Make sure the snaps bend backwards (away from the side with chamfer) while the AIM chip is being pressed down.

## AIM CHIP REMOVAL TIMING 2 min

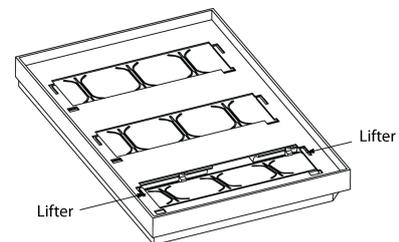
### MATERIALS

#### Others

- AIM Holder with assembled AIM Chips

4. 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.
5. Hold the AIM holder on the long sides with both hands.
6. Place your index fingers below the lifters and push the lifters up. The snaps will first dislodge from the indexes but still grab on the long side of the AIM chip.

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



For illustration of lifters only. **Do not** flip the holder while removing AIM chips.

7. Continue to push gently until the long side of the AIM chip is free from the snaps. You may place your thumbs at the corners of the AIM chip to prevent it from flipping. Remove the AIM chip.

**Reminder** Repetitive assembly and removal of chips may cause fatigue of the snaps thus breaking them.

**! Critical** Abrupt force exertion on the lifters may cause the AIM chip 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 typical percent loss of medium over 24 h from an AIM chip (with 100  $\mu$ l of medium in each media channel) on a AIM holder is 10 % without any humidity control measures and this can be reduced to 6 % with the following steps applied.

There are 8 inbuilt reservoirs on the AIM holder: 4 larger reservoirs are on the side opposite to the chamfers while 4 smaller reservoirs are on the same side as the chamfers.

### MATERIALS

#### Reagents

- Sterilized water

#### Others

- AIM Holders with assembled AIM Chips

- 
8. Add 1 ml of sterilized water into each of the larger reservoirs while 0.5 ml of sterilized water into each of the smaller reservoirs.
  9. Cover AIM holders with the dedicated covers.
  10. 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** 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.

**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.

**Reminder** Increase the volume of culture medium per media channel from 100  $\mu$ l to 150  $\mu$ l can help reduce the percent loss of medium from 6% to 5%. Any further increase in the volume of culture medium will not be beneficial in minimizing the evaporation rate.