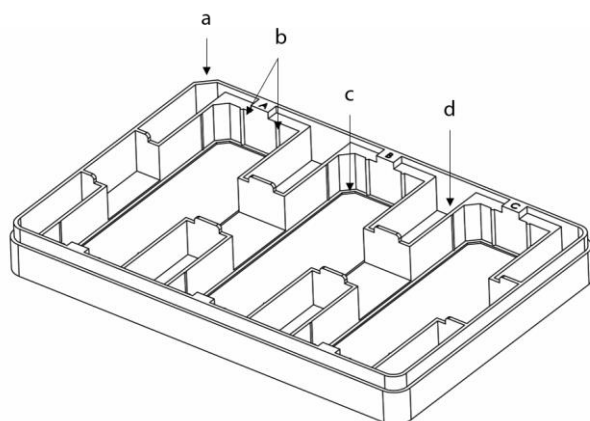


INTRODUCTION

HOL-2 Chip Holder is a sterile, single use, stackable holder that can fit up to three AIM idenTx 3 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 idenTx 3 chips onto/from the idenTx Holder as well as the humidity control measures within the idenTx Holder.

SCHEMATIC

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



Nomenclature:

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

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IDENTX 3 ASSEMBLY **TIMING** 2 min

MATERIALS.

- idenTx Holder
- idenTx 3

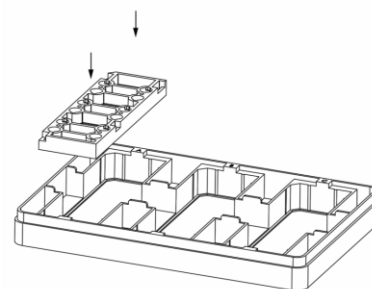
Others

- 37°C incubator

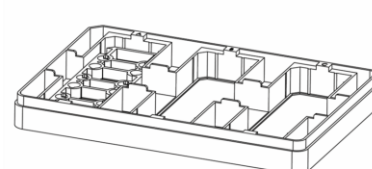
1. Align idenTx 3 to the idenTx Holder by matching their chamfers.
2. Push idenTx 3 downward until it is flush against the ledge. The tightening bumps provide sufficient resistance to hold the chip in place.

! Critical Avoid touching the media ports and gel inlets when pushing the idenTx 3 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

IDENTX 3 REMOVAL TIMING 2 min

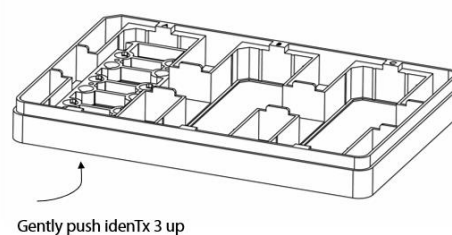
MATERIALS

- idenTx Holder with assembled idenTx 3

- Remove water from all reservoirs (if any) and medium from all ports of the idenTx 3 that is going to be removed from the holder.
- Hold the idenTx Holder on the long sides with both hands.
- Place your index fingers and thumbs on the bottom and the top of the idenTx 3, respectively.
- Exert a gentle upward force with the index fingers to lift the idenTx 3. Once the idenTx 3 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.



! Critical Abrupt force exertion on the idenTx 3 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 idenTx Holder houses reservoirs to create a humidified environment to limit evaporation from idenTx 3 to as low as 1% over 24 hours when kept in a CO₂ incubator.

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

MATERIALS

Reagents

- Sterilized water

Others

- idenTx Holders with assembled idenTx 3

-
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 idenTx 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 idenTx holders in an incubator, the model of the incubator and the frequency of the incubator being accessed.