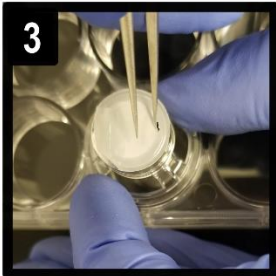


A**1**

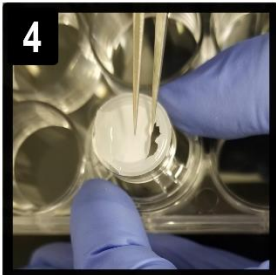
To remove the membrane from a culture insert, begin by placing the VHSE culture insert upside down, over a well.

**2**

Stabilize each culture insert using one hand & hold fine tip forceps or a precision knife with the other hand.

**3**

Use the tool to make a small incision on the membrane at the circumference border of the insert well. Start in an area that is not close to the VHSE culture.

**4**

Using the tool, lift the membrane up & begin to pull toward the opposite side. More incisions may be needed. Avoid puncturing the construct.

**5**

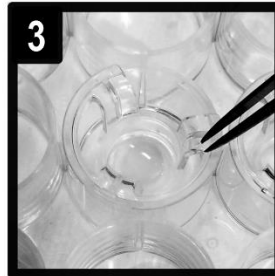
Pull the membrane off from other edges as well. The construct should fall into the well below, if it doesn't then gently separate the construct from the membrane using a tool.

B**1**

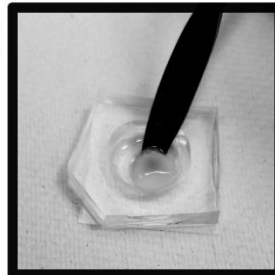
Keep each VHSE submerged in staining fluid or PBS by using the culture insert housing. All membrane pieces should already be removed.

**2**

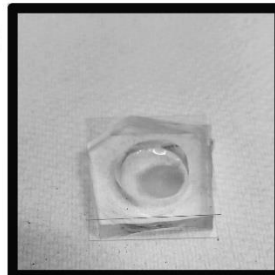
The insert housing keeps constructs from floating during staining; it pins down the construct without affecting macro-structure.

**3**

Each VHSE should be totally submerged when staining. For long term storage, allow the construct to sit in the middle of the well with plenty of PBS.

C

The safest way to move a construct is with a small round tipped scoopula to prevent accidental squeezing or puncture from fine-tip forceps.

D

A VHSE is shown in a PDMS well constructed for imaging. PDMS is glued to a glass slide, creating a viewing window. PBS is in the well as imaging fluid. A glass cover slip is placed over the well.