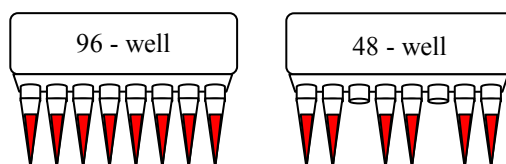


## HμREL® Hepatic Co-culture System Maintenance Protocol

### Maintenance:

#### Medium should be replaced ever 48 hours

1. Warm HμREL PlatinumHeps™ Maintenance Media to 37°C using a waterbath or incubator.
2. After medium is warmed, transfer your HμREL co-culture plate from the incubator to a biosafety cabinet.
3. For 96-well and 48-well plates:
  - 3a. Use a multichannel aspirator and micropipetter with the following tip configurations:



- 3b. We recommend aspirating and replacing medium 3 columns at a time to ensure cells do not dry out hence functionality and viability are maintained
4. For 24-well and 12-well plates:
  - 4a. Use a single channel aspirator tip and micropipetter
  - 4b. We recommend aspirating and replacing medium 3 wells at a time to ensure cells do not dry out hence functionality and viability are maintained
5. Refer to the table below for our recommended medium volume/well.

### **Important Considerations:**

- Medium aspiration should be performed using a low vacuum only.
- For aspiration, slowly move aspirator tip(s) down against the side of the well.
- Aspirator tips should NEVER touch the bottom of the wells.
- Change 2 plates at a time, extended amounts of time the plates are outside the incubator should be limited for optimal cellular function
- For experiments, DMSO concentrations should NOT exceed 0.5%

Well Plate	Volume/Well
96-Well	80 μL
48-Well	150 μL
24-Well	400 μL
12-Well	1000 μL