Measuring Trans Epithelial Electrical Resistances (TEER)

1. Using a EVOM voltohmmeter from World Precision Instruments, sterilize the STX2 electrode probe ("chopsticks") in ethanol for no more than 5 minutes. Remove and allow to air dry.
2. Wash off ethanol/residue left on probe with media or sterile buffer solution; be careful not to ignore ethanol droplets higher on the probe that might later fall into the transwell. When washing off ethanol residue with sterile solution, be sure the wash solution does not fall "up" towards the wire-end of the "chopsticks" since this means the media will then touch the unsterile part of the chopsticks and could then fall back down the chopsticks and contaminate the well when measuring TEER.
3. Perform a test of the meter (Test button) to make sure it is calibrated accurately; display should read 1.00 or 1000 depending on whether you are on the 2000ohm or 20k ohm scale. Flip the meter to the 2000 or 20k ohm scale, depending on the anticipated resistances of the Transwells you are measuring.
4. TEER is temperature sensitive; since conductance increases at higher temperatures, TEER will decrease (and the longer the plate is out of the incubator, conversely, the TEER will increase). Make sure to leave the plate inside the incubator until ready to measure all the wells immediately.
5. Lay the plate flat in the tissue culture hood and hold the probe perpendicular while inserting it in. If the plate is tilted, the meniscus in the apical and basolateral chambers will be uneven from well to well, and may often not cover the entirety of the electrode.
6. Ensure the basolateral electrode is touching the bottom of the well and the apical electrode is well away from the Transwell membrane before recording the value.
7. Apply the same method to all the wells; preferably do not widen the electrodes once inside the well. **It is critical that each Transwell is measured the same way (basolateral probe touching well bottom, with the same angle of approach for the STX2 electrode/chopstick in each well) to avoid significant variability in TEER measurements across wells. Never measure TEER with the Transwell "stuck" on the one stick of the STX2 electrode and elevated above the receiver plate.**
8. To prevent cross-contamination, be sure to place the STX2 electrode in ethanol for 2-5 minutes in between each group of plates, and also between any change in RPE type (e.g. when going from fetal to adult RPE, or from human to porcine RPE, or from primary culture to cell line). Repeat steps (1) and (2) above to wash the ethanol off the electrode.

<u>Notes:</u>

PBS will have very low TEER due to high conductance. MilliQ water will max out the display due to very low conductance. The Transwell membrane itself has some resistance e.g. ~100 ohm * cm^2 per Transwell in a 24-well plate. Resistance will be lower for membranes in 12- and 6-well Transwell plates due to larger cross-sectional area and higher conductance.

To calculate TEER, the surface area of the transwell (in cm^2) is multiplied by the NET resistance (which is the resistance measured minus the resistance of a blank Transwell covered by cell culture media. For example, if a 24-well Transwell, which has a surface area of $0.33cm^2$, has a resistance of 600 ohms, and a blank Transwell covered in media has a resistance of 100 ohms, then the TEER for this well would be: 600-100 = 500 net ohms * $0.33cm^2$ = 165 ohm*cm^2.

It can be tricky trying to get the STX2 electrode into the Transwell without it "sticking" to the Transwell. A very gentle angulation towards the center of the transwell as the electrode is first inserted, followed by a "straightening out" of the electrode so it is perpendicular to the plate as the electrode is dropped down further, can work well. The electrode should be perpendicular as it approaches the bottom of the plate and the basolateral probe hits the receiver plate bottom. To remove the electrode without sticking, pull it directly up and then once the electrode is no longer touching the receiver plate bottom, angle the electrode towards the center of the well to help it slide past the Transwell basolateral openings.