ACE-2 Expression

1. **TEER measurement before testing for ACE expression**
   a. Check TEER of activated BBB Make sure is TEER greater than 150Ω x cm²
   b. Model is a 12 Well : 12 well TEER (Ω x cm²) = (Total R - Blank R) x 0.33

Blank R Measurement:

Probe reading in 1XPBS Probe and blank insert reading.
807-247 = 560

560 = Blank R

c. **Raw Data:**
   i. Insert 1.

   \[
   1238 - 560 = 678 \times 0.33 = 223.74 \Omega \times \text{cm}^2
   \]

   ii. \( 1244 - 560 = 684 \times 0.33 = 225.72 \Omega \times \text{cm}^2 \)
iii. $1381 - 560 = 821 \times 0.33 = 270.93 \, \Omega \times \text{cm}^2$

iv. $1237 - 560 = 677 \times 0.33 = 223.41 \, \Omega \times \text{cm}^2$

v. $1062 - 560 = 502 \times 0.33 = 165.66 \, \Omega \times \text{cm}^2$

vi. $1244 - 560 = 684 \times 0.33 = 225.72 \, \Omega \times \text{cm}^2$
d. Results: The minimum TEER value to activate the BBB should be greater than 150Ω x cm². The lowest number we got when testing all six inserts to be tested was 165.66 Ω x cm². We had a range of 165.66 Ω x cm² to 270.93 Ω x cm².

2. **ACE-2 Staining**
   a. Membranes with fixed cells were removed from the inserts into PBS.
   b. Blocked with antibody blocking solution (cat no. SF40011) for 30 min RT followed by incubation with a permeabilization solution (cat no. SF40012) for 20 min RT.
   c. Inserts were placed in 6-well plate and incubated with primary anti-ACE2 antibody (AF933, R&D Systems) at 5 ug/mL overnight at 4 degrees C.
   d. The membranes were washed 3 times 15 min each in PBS.
   e. Incubated with anti-goat Cy3 secondaries (Jackson ImmunoResearch) for 30 min RT.
   f. Washed 3 times 15 min each in PBS, placed onto histological slides and mounted under iBright (cat no. SF40000-10) with DAPI to counter stain cell nuclei.
   g. Collected images on FL microscope.

*Note: Can be tricky to remove and handle the membranes with cells so they do not become crooked.*

ACE-2 staining (red) and DAPI nuclear counterstain (blue) of the endothelial cells on the bottom of the inserts. Images collected on a microscope.