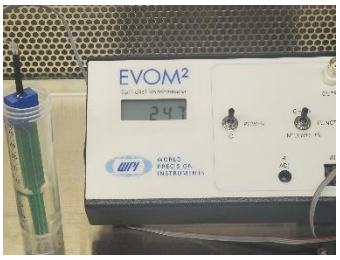
NEUROMICS

A Division of CA3 Biosciences, Inc.

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 cm^2
   b. Model is a 12 Well : 12 well TEER (Ω x cm^2) = (Total R - Blank R) x 0.33

   Blank R Measurement:



Probe reading in 1XPBS

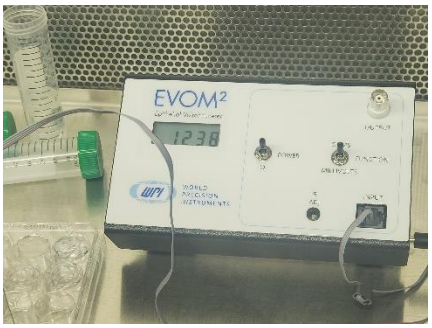
Probe and blank insert reading.

807-247 = 560

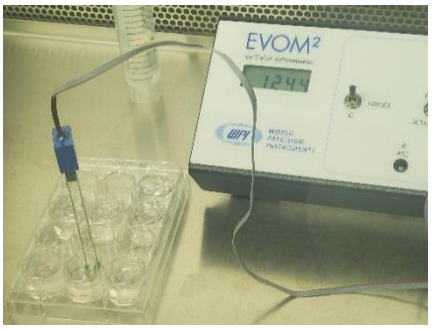
*

560 = Blank R

   c. Raw Data:
      i. Insert 1.
      1238 -560 = 678 x 0.33 = 223.74 Ω x cm^2



      ii. 1244 -560 = 684 X 0.33 = 225.72 Ω x cm^2



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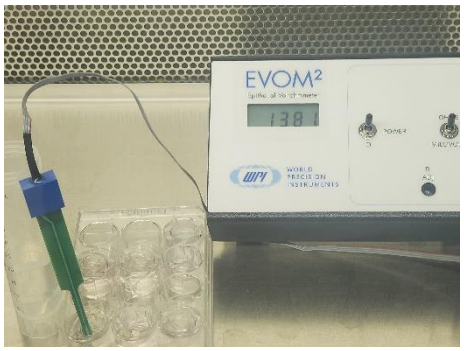
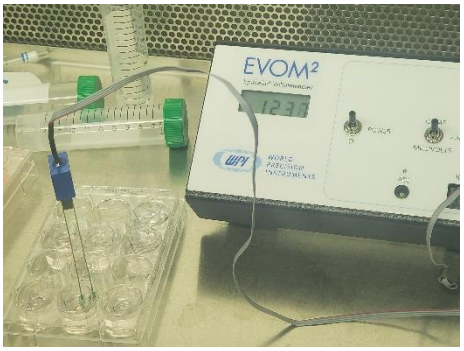
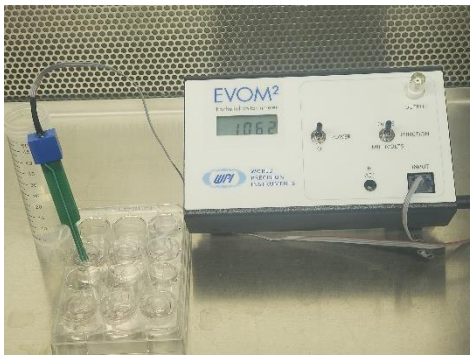
iii. 1381-560 = 821 x 0.33 = 270.93 Ω x cm^2



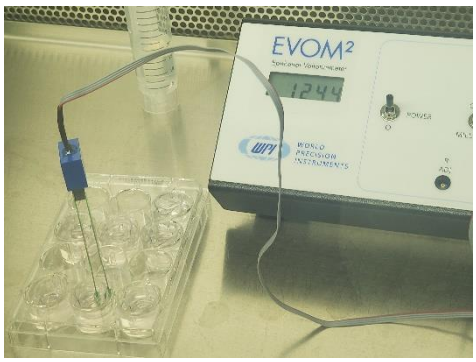
iv. 1237-560 = 677 x 0.33 = 223.41 Ω x cm^2



v. 1062 – 560 = 502 x 0.33 = 165.66 Ω x cm^2



vi. 1244 – 560 = 684 x 0.33 = 225.72 Ω x cm^2



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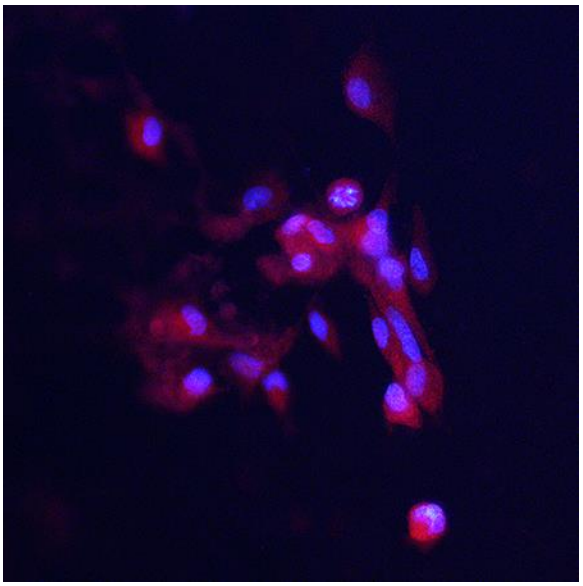
d. Results: The minimum TEER value to activate the BBB should be greater than 150Ω x cm2. The lowest number we got when testing all six inserts to be tested was 165.66 Ω x cm^2. We had a range of 165.66 Ω x cm^2 to 270.93 Ω x cm^2.

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.