ANGIOTEER

User Guide

Organo Biotech

Simple Platform for Complex Biology

Congratulations on your purchase of an AngioTEER™ by OrganoBiotech. Please read this user guide which explains proper operation. This manual is also posted on our website, www.organo-biotech.com. We're confident that your AngioTEER will become an essential tool in your laboratory and we wish you success with your work.

1. INTRODUCTION

While dextran permeability assay can provide information on how effectively the barrier prevents the transport of molecules of specific sizes, Trans Epithelial Electrical Resistance (TEER) measurements can offer non-invasive monitoring across various time points during experiments. However, conventional TEER sensor lacks high throughput capabilities, posing challenges in measuring larger sample size. Moreover, their manual measurement process can introduce human bias and error into the results. To overcome these limitations of conventional TEER meter, we introduce **AngioTEER**, a high-throughput and automated TEER meter (**Figure 1**).

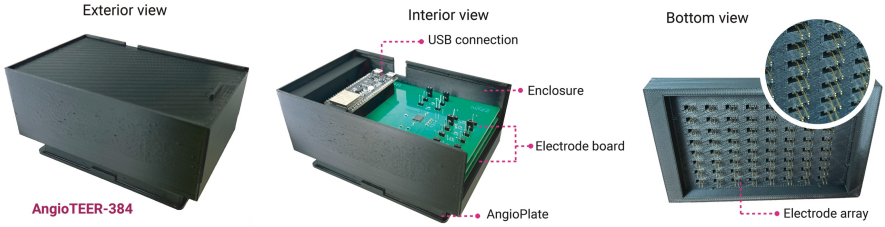

Figure 1. AngioTEER internal components

AngioTEER consists of two main components: an electrode board housing an array of gold-plated electrode pairs, and an enclosure designed to encase the electrode board for ease of use. The electrode board is equipped with a built-in USB power cable and Wi-Fi connection, enabling users to easily power and access the device through Wi-Fi connection and the AngioTEER software, which can automatically record the measured values in real-time. The entire device was designed to be compatible with AngioPlate and function as a lid for seamless integration. The electrodes were

aligned so that when the AngioTEER is placed on the AngioPlate, one pair is inserted into the inlet well, and the other pair is placed in the middle/tissue well (**Figure 2)**. In this four-lead topology setup, one pair applies a known electrical current to the system, while the other pair of electrodes measures the resulting voltage from which total electrical resistance can be measured and is displayed on the screen.

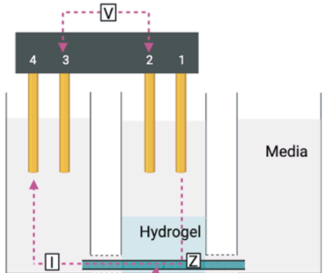

Figure 2. Electrode placement in AngioPlate

2. MATERIALS

- AngioTEER (Included)
- USB power cable (Included)
- AngioTEER Software (download on www.organo-biotech.com)
- Warming plate, F37015-3000, SP BEL-ART (recommended, but optional)
- AngioPlate, A005, OrganoBiotech (Not included)
- UVclave Chamber, Benchmark Scientific B1450 (Not included)
- Computer, Windows or Mac operational system (Not included)
- Standard 384-well plate, VWR 10814-224 (Not included)

3. ANGIOTEER FEATURES

- Default detection range: 3000-10000 ohm
- Adjustable detection range: 50-200,000 ohm
- Data collection from 128 tissue units on AngioPlate in default and flipped modes
- Continuous measurement of 64 tissue units on AngioPlate in default mode
- Wireless data transfer
- Compatible with AngioPlate384, UniPlate384 (in development), and IFlowRocker

4. SPECIFICATION

- Power Requirement: 24 VDC, 2.5 Amp
- Relative Humidity: 5 – 90% non-condensing
- Operating Temperature: 4 – 40°C
- Altitude: <2000m
- Storage Temperature: 4 – 40°C

5. SOFTWARE INSTALLATION

1. Download python 3 and enable IDLE from this link, https://www.python.org/downloads/
2. After you download the software package received via email after purchase, open terminal or command central and type the following and press enter to install these addons,

- To install Webstock type: pip3 install websocket-client
- To install Numpy type: pip3 install numpy
- To install Matplotlib type:pip3 install matplotlib
- To install Flet type: pip3 install flet
- To install DateTime type: pip3 install DateTime

3. Download the AngioTEER software from this link provided in the email.
4. Open the file angioTEER.py that has been provided in the folder
5. Software installation is now complete. The buttons will be greyed out until the device wifi is connected (refer next section).

Reach out to customer care at info@organo-biotech.com if any additional support is needed during software installation.

6. ANGIOTEER INSTALLATION AND CONNECTION

1. Connect the USB from TEER to a laptop
2. Open the angioteer.py file
3. Make sure the python code window is selected and then select “Run” from the toolbar and select “Run module”

from the drop-down menu which starts the program within few seconds (**Figure 3**)

4. Connect to the AngioTEER wifi on your laptop (password is OrganoBiotech)
5. Once the wifi is connected, the greyed out wifi button turns to a red button indicating that the TEER device is ready for use.
6. Select the Pick folder option to select a desired location to save the generated csv file.
7. Select desired wells to be measured by clicking on the well or select whole plate using the select all option on the screen.

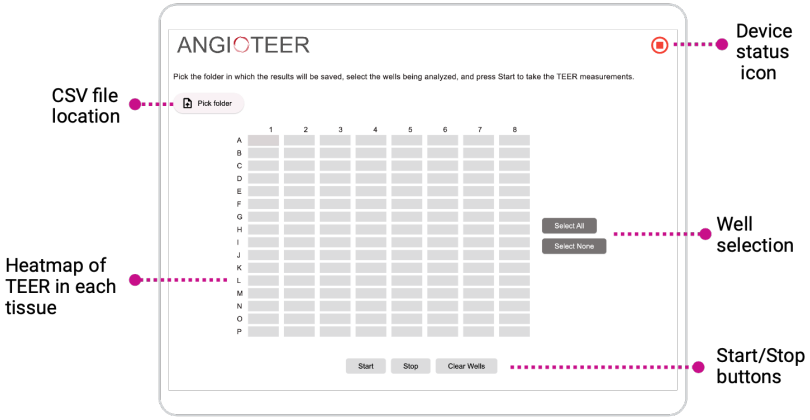

Figure 3. User interface

8. Select Start when you are ready to start the measurements.
9. The teer is set to read A, C, E, G, I, K, M, O rows in the default mode and rows B, D, F, H, J , L, N, P when the flipped mode is ON (**Figure 4**).
10. The system is in default mode when the USB connection is facing you; The system is in flipped mode when the USB connection is facing away from you.
11. When the system prompts you to flip the plate to continue reading, change the orientation of the AngioTEER as mentioned above and click OK to continue with the measurement of remaining wells after you have rotated the device.

12. Once the TEER reads all the desired wells, you can stop the read.
13. The program automatically creates a CSV file with the raw ohm values which can be used to calculate TEER values.

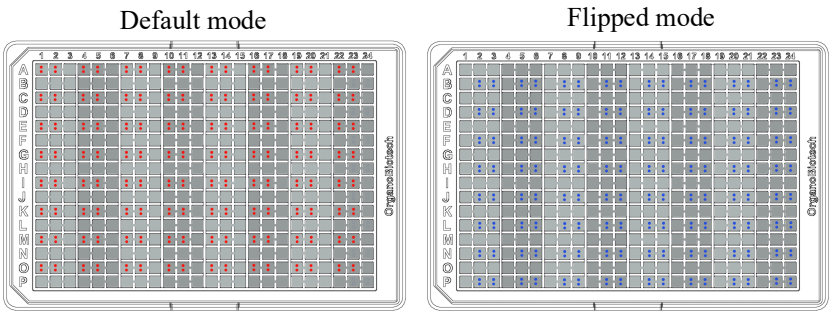

Figure 4. Electrode placement in two modes is shown. The red and blue dots indicate the positions of the electrodes. Each tissue unit is equipped with four electrodes. Rotating the AngioTEER by 180 degrees will alternate between default and flipped mode. By alternating between the modes, measurements can be obtained from all 128 tissue units.

7. ANGIOTEER OPERATION

AngioTEER sterilization and preparation:

1. Sterilize the electrode side of the TEER device under UV inside the recommended UVclave chamber for 30 minutes
2. Prepare three standard 384 well plates. The first plate contains 90 µL of sterile distilled water in each well.
3. The second plate contains 90 µL of PBS in each well.
4. The third plate will be an empty plate.
5. Place the UV sterilized TEER device with electrodes on the 384 well plate containing PBS for 5 minutes.
   IMPORTANT: Washing with PBS will help cool down the electrodes and clean the surface prior to use.
6. The well plate containing PBS can be used to wash the electrodes whenever the electrodes are moved to a different well or a different plate to minimize cross-contamination.
7. At the end of each use, wash the TEER electrodes with distilled water to remove PBS and then dry in the empty plate and stored for future use.

IMPORTANT: Washing with distilled water helps remove salt particles from PBS prior to drying.

8. Repeat these steps each time before using the device.

AngioPlate preparation:

9. Follow AngioPlate protocol to setup the experiment
10. Make sure to include no cell controls/acellular controls (n=4-5) when performing TEER.
11. Warm culture media for 15-20 minutes
12. Change culture media for all wells with 90 mL of media in the inlet and outlet well and 65 mL in the middle well. Maintaining equal liquid level in all three wells and changing culture media before each read is important as TEER is sensitive to volume changes, temperature and pH.
13. Once the media is completed, keep the plate leveled under static condition inside an incubator maintained at 37°C for 15 minutes to allow for liquid volumes to equilibrate.
14. Your AngioPlate is now ready for TEER measurement

TEER measurement in AngioPlate:

15. Replace the AngioPlate lid with TEER device.
16. Place the plate with AngioTEER on the warming plate (recommended) or inside the biosafety cabinet while measuring TEER.
17. Refer to installation section on how to open the software
18. Pick a folder, select the desired wells to be measured once the software opens and start the measurement
19. Data is collected as CSV file in the folder selected before the read.
20. If you want to use the flipped mode, make sure to immerse the electrodes in the 384-well plate containing PBS to wash the electrodes before flipping the device.
21. Once all readings are taken, immerse the electrodes in PBS for 5 minutes and then distilled water for 5 minutes and then on the empty 384-well plate to dry for 15 minutes and store.
22. Change culture media again in all wells after TEER measurement is highly recommended.

IMPORTANT: TEER electrodes could leave behind residual reactive oxygen species during operation which can be damaging to the cells if not removed properly. A media change will prevent this issue.

TEER Data analysis:

1. Average the raw ohm values of no cell controls (blank controls) and subtract it from the raw ohm values of tissues.
2. Multiply this value with area of tissue exposed to the current (AngioPlate single channel, cat#A002: 0.0106 cm^2, IFlowPlate barrier cat#A001-2: 0.1369 cm^2) to calculate TEER (ohm cm^2)

   For example,
   TEER = (Total Electrical Resistance – Average Blank Resistance) x Area

   For single channel AngioPLate384,

   TEER = (8989 – 6608) x 0.0106
   = 25.24 Ω cm^2

8. MAINTENANCE

1. Before and after each use, always make sure to wash/rinse the electrodes by immersing them in a 384-well plate containing PBS and then distilled water.
2. Make sure to let the electrodes dry before storing them.
3. Always store the device by placing them over a clean standard 384-well plate to protect the electrodes.

Recommended monthly maintenance:

1. The electrodes may have some calcium deposits with regular use, this can be cleaned with 2% Citranox (Alconox, #cat. 1832-1) in distilled water.
2. Heat the solution at 60°C and immerse the electrodes for 30 minutes in 90 µL of cleaning solution

3. After this, wash the electrodes by immersing them in a well plate containing distilled water to remove any residual citronox solution for 5 minutes.
4. Refresh the distilled water and immerse the electrodes again for another 5 minutes to ensure the residual cleaning solution is washed away.
5. Immerse the electrodes in 70% ethanol in a 384-well plate and then dry before storing.

9. TROUBLESHOOTING

1. The ohm values for no cell control ranges from 4000-5000 ohms. If the reading lower than normal (e.g., 300-400 ohms) then likely your electrodes aren't positioned properly. Make sure to align the plate properly to ensure that one pair goes into the inlet and the other pair goes into the middle well.
2. If the TEER reading is higher than normal (e.g., 8000-1000 ohms), then likely there is an air bubble trapped inside the inlet/outlet channels that interfere with the electrical current. Try gently aspirate out the air bubbles from the inlet/outlet wells and read again
3. If the software is non-responsive after multiple reads, close and restart the program.

10. WARRANTY

OrganoBiotech warrants its Products against defects in materials and workmanship for time periods which vary according to the Product. Within these time periods, OrganoBiotech will replace or repair, without charge to the original purchaser, any part which is defective. The AngioTEER warranty is one year. The warranty is void if the Product is defective due to product accident, product modification, exposure to radiation, connection to an improper electrical supply, lack of proper maintenance, contamination, improper installation or misuse. If the product is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired. The warranty shall also not apply to

defects arising from fire, flood, lightning or other conditions unrelated to correct operation of the Product.

OrganoBiotech's liability is limited, at the company's election, to (1) repair of the Product, or (2) replacement of the Product or defective parts. Evidence of purchase by the original purchaser is required. OrganoBiotech may also request documentation of proper maintenance, if applicable. Operator is responsible for: providing proof of purchase and providing normal care and maintenance.

11. WARNINGS AND CAUTIONS

Read the user's manual before operating. Do not put excessive weight on the platform. Do not insert fingers or objects into the platform. When working with hazardous or pathogenic samples, operate the AngioTEER in a biosafety cabinet or other standard laboratory safety enclosure. No user serviceable parts are inside of the instrument. For indoor use only. Pollution Degree 2 per EN 61010-1. Overvoltage Category II per EN 61010- 1. Enclosure Protection: Not Protected Against the Ingress of Moisture. Do not immerse in liquid. Before touching the AngioTEER touch a bare metal surface to discharge static electricity.

12. DISCLAIMER

OrganoBiotech makes no other warranty, expressed or implied, with respect to its Products. ORGANOBIOTECH MAKES NO WARRANTY RESPECTING THE MERCHANTABILITY OF THE PRODUCTS OR THEIR SUITABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE OR USE. In no event shall OrganoBiotech be liable for indirect, special, incidental or consequential damages of any nature. OrganoBiotech Inc is not liable for any damages, including but not limited to, lost profits, lost savings, or other incidental or consequential damages arising from ownership or use of this product, or for any delay in the performance of its obligations under the warranty due to causes beyond its control. Any recovery for any claim shall be limited to the original purchase price for the product.

OrganoBiotech also reserves the right to make any improvements or modifications to the product described in this manual at any time, without notice of these changes. OrganoBiotech products are not designed, intended, or authorized for use in applications or as system components intended to support or sustain human life, as a clinical medical device for humans, or for any application in which the failure of the product could create a situation where personal injury or death may occur.

13. CONTACT INFO

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