

## **VO<sub>2</sub> Treadmill Protocol V1.5 (FBRI)**

This is a protocol for measurement of VO<sub>2</sub>max in mice during treadmill running, which was modified from Columbus Instruments' manual and Lee-Young et al. (Wasserman) JBC, 2009; 284:23925-34.

### **Procedure**

1. Pre-running: **Acclimate mice** to the treadmill by running for 10 min at 10 m/min for 3 consecutive days during the beginning of the light cycle (0700/1900) prior to performing the VO<sub>2</sub>max test.
  2. The evening before the test
    - **Lock the wheel** of any exercising cages the evening prior to the test. Fasting is NOT needed.
    - **Turn on the power** (surge protector) behind the treadmill to turn on the entire system and allow it to “warm up” can take up to 2 hours but best to have it set the night before.
  3. The morning of the test:
    - a. **Begin the test** at the turn of the light cycle (0700)
    - b. **Check Drierite** (should be blue). If purple, change it and save purple Drierite for reuse.
    - c. Plug in the USB that is connected to the oxymax treadmills.
    - d. **Open the Columbus instruments program (CL-link)** pictured below (**Figure 1**) and open the setup experiment tab. Given that the correct USB cord is plugged in the treadmill (1) and oxymax (2) options should be present.
    - e. When software is ready to be calibrated, **open the tank valve** (gray knob on top of the tank), do not adjust any other settings on the tank, should read 10-15. If low order another tank.
    - f. If the warm up has occurred overnight the system should be ready for calibration, **click start calibration** (3). This will menu will show the time and progress that calibration takes, ~8 min. After calibration close the tank valve. (If calibration failed, check oxygen sensor's production date, each sensor lasts~6 months)
    - g. While calibration is occurring, **weigh mice**. This is vital because VO<sub>2</sub> outcome is ml/kg/hr.
    - h. **Edit the experiment** name and file location and name with the date and experimental codes (4).
    - i. **Select 3 cages** in the Experiment setup tab on the right side on the creen for the oxymax to samples gases. Importantly, if 4 cages are selected, it will rotate through all 4 cages.
    - j. On the setup experiment tab click on the **edit clip board** (5) to bring up the edit subject information menu (**Figure 2**). Add or remove subjects as necessary (3 max). Input the experimental code for the name, enter weight, strain, gender, and date of birth.
    - k. **Drag the subjects** to lanes 1, 2, and 3 pictured in **Figure 2** and confirm that those boxes are checked in the experiment set up tab of the setup experiment tab (6). If the three boxes aren't checked it will not take measurements in the three lanes.
  1. **Make treadmill protocol** by clicking on the treadmill tab (7) and set up the experiment according to **Table 1**.
  - m. **Load mice** into chambers and turn on shock grids. Tighten chambers to valves if necessary.
4. **Start the run** and monitor running activities closely. If a mouse stops running and stays on the electrical grid for more than 5 seconds continuously, turn off the shock grid. Allow the recording for 2 more cycles (~7 minutes), do not open then chamber. If it is the last animal wait for 2 additional recordings and stop the run.
5. **Save the run** by clicking on export CSV (8) from the analysis tab as shown in **Figure 3** to bring up the export CSV data window. Click on the data that you want to export (9) and the subjects you want to export (10). These data will be saved to individual excel files when you click export (11).

Figure 1.

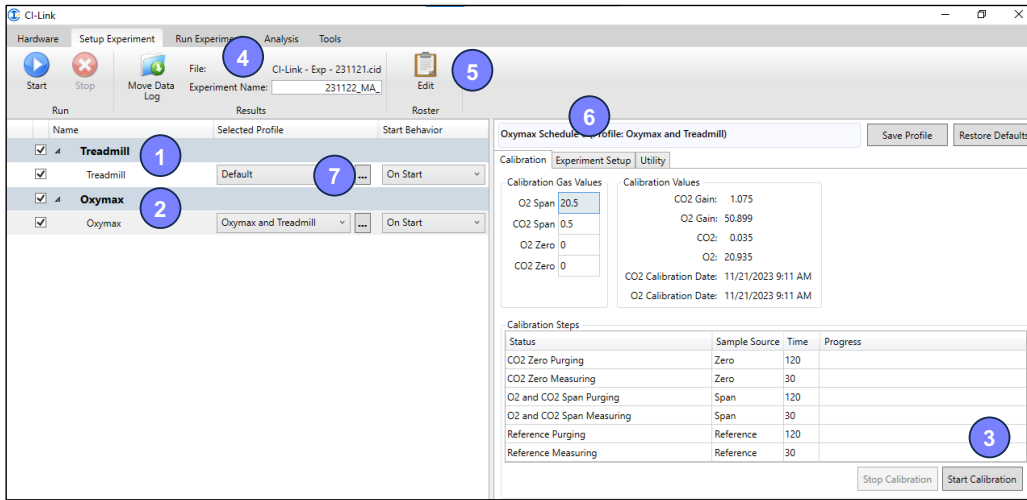


Figure 2

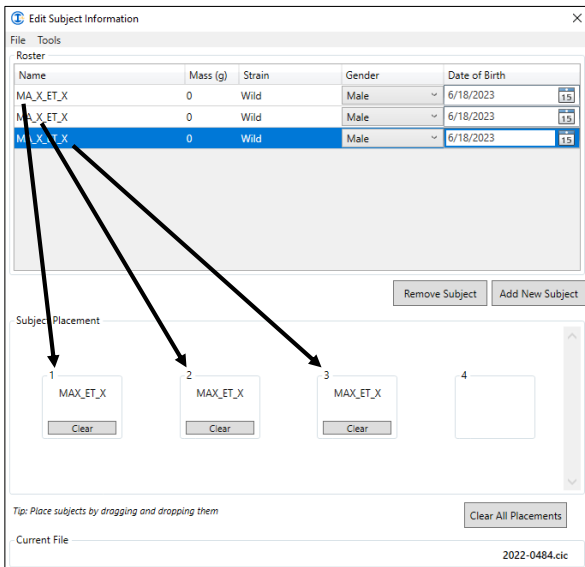
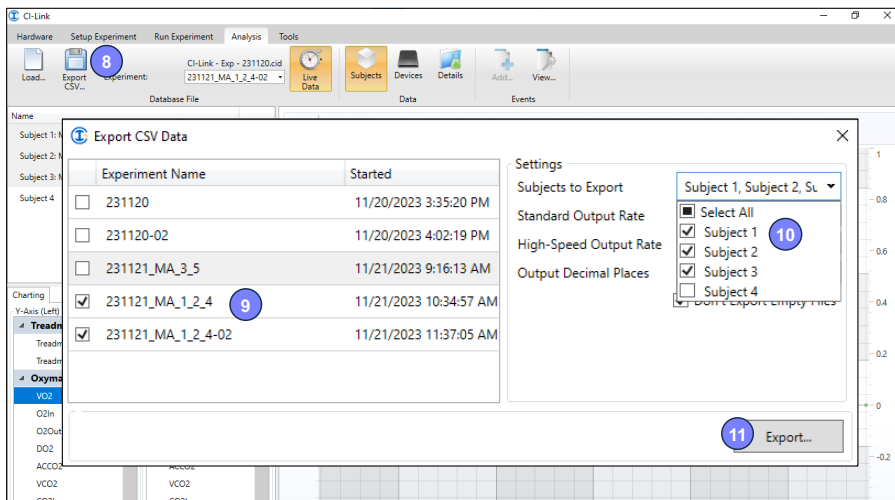


Figure 3



<b>Step</b>	<b>Stage Time</b>	<b>Time (min)</b>	<b>Speed (m/min)</b>	<b>Incline</b>
1	00:02:00	-14	0	5°
2	00:12:00	-12	5	5°
3	00:03:00	0	10	5°
4	00:04:00	3	13	5°
5	00:03:00	7	16	5°
6	00:03:00	10	19	5°
7	00:03:00	13	22	5°
8	00:03:00	16	25	5°
9	00:03:00	19	28	5°
10	00:03:00	22	31	5°
11	00:03:00	25	34	5°
12	00:03:00	28	37	5°
13	00:03:00	31	40	5°
14	00:03:00	34	43	5°
15	00:03:00	37	46	5°
16	00:03:00	40	49	5°
17	00:03:00	43	52	5°
18	00:03:00	46	55	5°
19	00:03:00	49	58	5°
20	00:03:00	52	61	5°
21	00:03:00	55	64	5°
22	00:03:00	58	67	5°
23	00:03:00	61	70	5°
24	00:03:00	64	73	5°
25	00:03:00	67	76	5°
26	00:03:00	70	79	5°
27	00:03:00	73	82	5°