## Treadmill Running Test for Mice.v7

This procedure will allow for measurement of endurance capacity in living mice repeatedly.

## Procedure

1. House mice in Treadmill Running Room \#18 (MR-4 Vivarium) in a dark-light cycle 8:20 pm off, 8:20 am on) for 1 week.
2. Prime the mice at $10: 00 \mathrm{am}$ on treadmill for 3 days ( 0.5 mph for 10 min ).
3. Perform the running test at 10 am on the $4^{\text {th }}$ day.
4. Perform a lactate measurement before starting the test (handle the mouse very gently and clip the very tip of the tail with scissors to get a little of blood (mice tend to bleed a lot during the test even with a small clip of the tail).
5. Run mice on $5 \%$ slope at 0.5 mph for 30 min .
6. Obtain a second measurement of blood lactate levels at 40 min of test by removing the mouse from the treadmill and milking its tail. Return the mouse to the treadmill immediately after the measurement.
7. Increase the speed by 0.1 mph every 30 min (not exceed maximal speed of 1 mph ) until exhaustion, which is defined as when mice refuse to run even when encouraged by brushing the tail 20 times for $\sim 20$ seconds. The signs of getting close to exhaustion are that mice have hard time to run to the front when encouraged and/or tail starts to fall.
8. Record the time of running for each mouse.
9. Obtain a final measurement of lactate from the tail. If it is clogged, just clip the very end of the tail with scissors again.

IMPORTANT: Do not stop the test if a mouse did not reach fatigue after 30 min at 1 mph (i.e. after 3 h of test). Proceed with the test but without further increment in speed.

## Calculation

Mph to $\mathrm{m} / \mathrm{min}$ conversion
$0.5 \mathrm{mph}=13.41 \mathrm{~m} / \mathrm{min}\left(0-30^{\prime}\right)$
$0.6 \mathrm{mph}=16.09 \mathrm{~m} / \mathrm{min}\left(30-60^{\prime}\right)$
$0.7 \mathrm{mph}=18.78 \mathrm{~m} / \mathrm{min}\left(60-90^{\prime}\right)$
$0.8 \mathrm{mph}=21.46 \mathrm{~m} / \mathrm{min}\left(90-120^{\prime}\right)$
$0.9 \mathrm{mph}=24.14 \mathrm{~m} / \mathrm{min}\left(120-150^{\prime}\right)$
$1.0 \mathrm{mph}=26.82 \mathrm{~m} / \mathrm{min}\left(>150^{\prime}\right)$
$30^{\prime}=402 \mathrm{~m}$
$60^{\prime}=885 \mathrm{~m}$
$90^{\prime}=1448 \mathrm{~m}$
$120^{\prime}=2092 \mathrm{~m}$
$150^{\prime}=2816 \mathrm{~m}$

To calculate the total distance:

For example, if the mouse runs for 125 min , the total distance would be $2093 \mathrm{~m}\left(120^{\prime}\right)+$ $5 \mathrm{~min} \times 24.14 \mathrm{~m} / \mathrm{min}=2214 \mathrm{~m}$

