3. Prepare enough Working Reagent (WR) for all reaction wells by transferring 20 µL dH2O to each well. 

2. Transfer 100 µL dH2O into each well. 

1. Transfer 100 µL dH2O into each well. 

Assay temperature (37°C is recommended). Briefly centrifuge tubes before use. All samples can be stored at –20 to –80°C for at least one month.

For adherent cells, do not harvest cells using proteolytic enzymes; rather, homogenize tissue (50 mg) in 200 µL cold PBS buffer. Centrifuge at 14,000 x g for 5 min at 4°C. Remove supernatant for assay. 

2. Linstad, RI et al (2013). Inhibition of Sorbitol Dehydrogenase by 


2. Transfer 20 µL dH2O into one well, this will be the blank. Transfer 20 µL of each sample into separate wells.

3. Prepare enough Working Reagent (WR) for all reaction wells by mixing, for each 96-well assay, 2 µL Substrate, 8 µL NAD/MTT Solution, 1 µL Diaphorase and 75 µL Assay Buffer. Add 80 µL WR to all sample and blank wells. Tap plate briefly to mix.

4. Incubate at desired temperature; read OD565nm at time 3 min (OD3) and time 15 min (ODs) on a plate reader.

CALCULATION

Subtract the OD3 from ODs for each sample well to compute the ∆ODs values, do the same for the blank to compute ∆ODb. SDH activity can then be calculated as follows:

\[
\text{SDH Activity} = \frac{\Delta OD_b - \Delta OD_s}{t (\text{min}) \cdot \text{Sample Vol (µL)}} \times n (\text{U/L})
\]

where \( \epsilon_{OD} \) is the molar absorption coefficient of reduced MTT. \( t \) is the light pathlength which is calculated from the calibrator. ODcal and ODbas are ODcal (OD3) values of the Calibrator and water. \( t \) is the difference in time between readings (15 min minus 3 min = 12 min is the recommended time). Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. \( n \) is the dilution factor.

Unit definition: 1 Unit (U) of SDH will catalyze the conversion of 1 µmole of D-sorbitol to fructose per min at pH 8.2.

Note: If sample SDH activity exceeds 125 U/L, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with SDH activity < 1 U/L, the reaction time can be extended to 2 hours. We recommend running kinetics and choosing two time points in which the activity remains linear.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and plate reader.

LITERATURE

