LDH Assay Protocol.v2

A modified protocol for quantification of LDH activity in serum or plasma according to abcam® ab102526 LDH Assay Kit (Colorimetric) protocol booklet

Procedure

1. Warm up the Assay Buffer to room temp and spin the vials prior to opening.
2. Add n x 2 µl of LDH Substrate Mix with n x 48 µl Assay Buffer to make Reaction Mix. (prepare a little bit more than you need considering everything in duplicates)
3. Add 0, 2, 4, 6, 8, 10 µl of the 1.25 mM NADH Standard Stock into 96-well plate in duplicate containing Assay Buffer to final volume of 50 µl (50, 48, 46, 44, 42, 40 µl Assay Buffer prior to the addition, respectively) to generate 0, 2.5, 5.0, 7.5, 10.0, 12.5 nmol/well NADH Standards.
4. Dilute 1.5 µl LDH Positive Control Stock in 13.5 µl Assay Buffer (1:9) to make LDH Positive Control.
5. Dilute serum or plasma (2 µl serum or plasma in 198 µl Assay Buffer; 100 times) to make sure the readings are within the range of the standard curve.
6. Keep samples and LDH Positive Control on ice during the assay.
7. Add 5 µl of LDH Postive Control in duplicates to 96-well plate containing 45 µl of Assay Buffer (added by a repeat pipetter).
8. Add 5 µl of diluted sample in duplicates to 96-well plate containing 45 µl of Assay Buffer.
9. Add 50 µl of the Reaction Mix to each well (Standards, Positive Control and samples) added by a repeat pipetter and mix well**.**
10. Read OD450 immediately as A1 (start the timer). Incubate at 37°C for 20 min (or longer if the LDH activity is low). Measure OD450 again as A2 (Record the incubation time starting at A1). Measure OD450 again at 30 min at A3 as a backup.

Data Analysis

1. Subtract the 0 nmol/well NADH background from Standard readings and plot NADH Standard Curve.
2. Use ΔOD450 (A2-A1) and NADH standard curve to calculate NADH in nmol (B).
3. LDH Activity = [B x Sample Dilution (100 in this case)] ÷ [20 min x V] = nmol/min/mL = mU/mL
4. V is the volume of diluted sample into the reaction well in ml (e.g. we add 5µl of diluted serum, so the V should be 0.005 ml).

Note

1. It is essential to read A1 and A2 in the reaction linear range. It will be more accurate if you observe the reactions progress. Choose A1 and A2 in the reaction linear range.
2. For Standard Curve, use A2 reading after 20 min incubation, do not subtract the A1 reading. The Standard reading is stable for a few hours.

Solution preparation

1. Dissolve LDH Substrate Mix (powder) in 1 ml of ddH_2O and store in 100 µl aliquot at -20°C.
2. Dissolve NADH Standard (powder) in 0.4 ml ddH_2O to generate 1.25 mM NADH Standard Stock. Aliquot the solution in 65 µl and store at -80°C.