

LDH Assay

Stock Solutions

Lactate (486 mg/10 ml) in 1 ml aliquots

INT (334 mg/10 ml) in 100 μ l aliquots

PMS (8.546 mg/10 ml DMSO) in 1 ml aliquots

NAD (86.142 mg/10 ml) in 1 ml aliquots

TRIS 0.2 M pH 8.2

Procedure

1. For a positive control lyse the cells of 3 wells by the addition of 0.1 % Triton-X for at least 30 min.
2. Remove 50 μ l of stimulated cell supernatant and transfer to a new 96 well plate.
3. Prepare the reagent by adding one aliquot each of lactate, INT, PMS, and NAD to 7 ml of TRIS buffer.
4. Add 50 μ l of reagent to the cell supernatant.
5. Measure the difference in optical density between 490 and 630 nm on a spectrophotometer.