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Malate Dehydrogenase Assay Kit (Cat #: E-124)

COMPONENTS: MDH Assay Solution- 5 ml (for 100 wells), store at -80ºC **(shield solution from light during assay)**
10x MDH Substrate- 0.5 ml, store at -80ºC
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The MDH enzyme activity assay is based on reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to INT-formazan, which exhibits an absorption maximum at 492 nm (molar extinction coefficient = 18 $mM^{-1}cm^{-1}$) and allows for measurement of MDH1 (cytosolic) and MDH2 (mitochondrial) activities in crude tissue/cell extracts and serum/plasma. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with dH_2O. Bring up ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 3 mg/ml. *Normalization of lysate protein concentration is recommended.*
4. Dilute serum/plasma with 1x Cell Lysis Solution 5-fold prior to assay.

Reagent thawing:

Keep thawed MDH Assay Solution and 10x MDH Substrate on ice. *Do not over thaw.* Shield MDH Assay Solution from light. Freeze solutions immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 10 parts of MDH Assay Solution, e.g. 50 µl dH_2O + 500 µl MDH Assay Solution.

Reaction solution is prepared by mixing 1 part of 10x MDH Substrate and 10 parts of MDH Assay Solution, e.g. 50 µl 10x MDH Substrate + 500 µl MDH Assay Solution.

Enzyme assay:

1. Thaw samples quickly and keep on ice. *Do not over thaw.* Add 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate (for control set and reaction set).
2. Add 50 µl control solution to one set of wells (control) and 50 µl reaction solution to the other set of wells (reaction). Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 30 min or 60 min. *Do not use CO_2 incubator*. Cherry red color should gradually appear in wells.
3. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.
4. If incubation for 30 min, MDH enzyme activity in IU/L = µmol/(L·min) = **ΔO.D.** × 1000 × 70 µl / (30 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 16.2**. If incubation for 60 min, MDH activity = **ΔO.D. x 8.1**.
Cell/tissue MDH enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101). Increase protein concentration or incubation time if detected enzyme activity is too low.

Additional information:

The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for material SDS information. 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.