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Succinate Dehydrogenase (SDH) Assay Kit (Cat #: E-161)

COMPONENTS: SDH Assay Solution- 5 ml (for 100 wells); store at -80ºC **(shield solution from light during assay)**
20x SDH Substrate- 0.25 ml, store at -80°C
10x Lysis Buffer- 25 ml, store at 4ºC (dilute 10-fold with ice-cold dH_2O)
10x Extraction Buffer- 20 ml, store at 4ºC (dilute 10-fold with ice-cold dH_2O)

PRODUCT DESCRIPTION: The SDH enzyme activity assay is based on reduction of the tetrazolium salt INT in a $FADH_2$-coupled reaction to formazan, which exhibits an absorption maximum at 492 nm ($\varepsilon = 18\ mM^{-1}cm^{-1}$), allowing for measurement of SDH activity present in cell/tissue extracts. Kit components are stable for several years if stored and handled properly.

Preparation of cell/tissue lysates:

1. **Cells**: Bring up ~10^6 freeze-thawed cells in ~0.1 ml ice-cold 1x Lysis Buffer by pipetting up and down. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH. Resuspend pellet (membrane fraction) in ~50 µl ice-cold 1x Extraction Buffer by pipetting up and down thoroughly, making sure the pellet is homogenized. Leave homogenate on ice for ~10 min with agitation. Repeat manual homogenization. Centrifuge homogenate at ~5000 rpm for 1 min. Harvest supernatant for SDH assay. Recommended sample protein concentration is 0.2 - 2 mg/ml.

2. **Tissue:** Mechanically homogenize freeze-thawed tissue in ice-cold 1x Lysis Buffer (~5 mg tissue in 0.5 ml). Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Collect supernatant for assays such as Hexokinase, GAPDH, and LDH. Resuspend pellet (membrane fraction) in ~0.25 ml ice-cold 1x Extraction Buffer by pipetting up and down thoroughly, making sure the pellet is homogenized. Leave homogenate on ice for ~10 min with agitation. Repeat manual homogenization. Centrifuge homogenate at ~5000 rpm for 1 min. Harvest supernatant for SDH assay. Recommended sample protein concentration is 0.2 - 2 mg/ml.

Preparation of control solution and reaction solution

Thaw SDH Assay Solution and 20x SDH Substrate and keep solutions on ice shielded from light. *Do not over thaw*. Prepare fresh control and assay solutions as follows:

Control solution is prepared by mixing 1 part of dH_2O and 20 parts of SDH Assay Solution, e.g. 25 µl dH_2O + 500 µl SDH Assay Solution.

Reaction solution is prepared by mixing 1 part of 20x SDH Substrate and 20 parts of SDH Assay Solution, e.g. 25 µl 20x SDH Substrate + 500 µl SDH Assay Solution.

Enzyme assay

1. Thaw protein samples and keep on ice. *Do not over thaw.* Pipette each sample up and down several times prior to sample addition. 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 sample wells and 50 µl reaction solution to the other set of sample wells. Gently agitate plate for 10 sec to mix contents. Cover plate and incubate in a 37°C incubator for 60 min or 90 min. *Do not use CO_2 incubator.*

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 60 min, SDH activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl/(60 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 8.1** (or **ΔO.D. × 5.4** for 90 min). SDH activity can be normalized by protein concentration or GAPDH activity (#E-101).

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.