BIOMEDICAL RESEARCH SERVICE CENTER

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Pyruvate Dehydrogenase (PDH) Assay Kit (Cat #: E-109)

COMPONENTS: PDH Assay Solution- 5 ml (100 wells); **store in aliquots shielded from light at -80ºC**
10x Cell Lysis Solution- 25 ml, store at 4ºC **(swirl bottle briefly prior to pipetting)**

PRODUCT DESCRIPTION: The enzyme activity assay is based on the reduction of the tetrazolium salt INT in a NADH-coupled reaction to INT-formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$) and allows for sensitive detection of PDH activity in crude samples. Assay solution is stable for several years if stored and handled properly.

Preparation of cell/tissue extract: Both soluble and membrane fractions can be used for PDH assay.

1. Wash at least 10^6 cells with ice-cold phosphate-buffered saline (PBS). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80ºC until use.
2. Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Add 50 – 100 µl 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. Centrifuge lysate in a refrigerated microfuge for 3 min at maximum speed (~13,000 rpm). Harvest supernatant for assay of soluble enzymes. Bring up pelleted fraction in 50 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently but thoroughly. Note that both the soluble and membrane fractions can be used for PDH activity assay.
3. For tissue extraction, weigh ~25 mg tissue and homogenize in 0.5 ml ice-cold 1x Cell Lysis Solution. Spin homogenate in a microfuge at ~5,000 rpm for 5 sec to deposit tissue debris. Transfer supernatant to another tube. Centrifuge supernatant in a refrigerated microfuge for 3 min at maximum speed (~13,000 rpm). Harvest supernatant for assay of soluble enzymes. Bring up pelleted fraction in ~200 µl ice-cold 1x Cell Lysis Solution by pipetting up and down thoroughly.
4. Perform protein assay to determine the protein concentration of both soluble and membrane fractions. Normalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution. A suggested protein concentration range is 0.5 – 2 mg/ml. Note: do not use a buffer containing reducing agents or SDS.

Enzyme assay for clear sample:

1. Thaw PDH Assay Solution and keep on ice. Briefly agitate solution prior to pipetting. Add 10 µl sample to a 96-well plate. Use 10 µl 1x Cell Lysis Solution as blank..
2. Reaction is initiated by adding 50 µl PDH Assay Solution to each well. Mix contents by brief gentle agitation. Cover plate and incubate at 37°C for 30 min or 120 min (for sample with low PDH activity).
3. Stop reaction by adding 50 µl 3% Acetic acid (not included in the kit) to each well followed by brief gentle agitation.
4. Measure $O.D._{492\ nm}$ using a plate reader. Subtract blank reading from sample reading. For 30 min incubation, PDH activity in IU/L unit = µmol/(L•min) = O.D. x 1000 x 110 µl / (30 min x 0.6 cm x 18 x 10 µl) = **O.D. x 33.95**. If incubation for 120 min, PDH activity in IU/L unit **= O.D. x 8.49**

Enzyme assay for hemolyzed sample:

1. Add 10 µl sample to each well of a 96-well plate in duplicate.
2. Add 50 µl dH_2O to one set of sample wells (sample color control well). Add 50 µl PDH Assay Solution to the other set of sample wells (reaction well). Mix contents by brief agitation. Cover plate and incubate at 37°C for 30 min or 120 min.
3. Stop reaction by adding 50 µl 3% Acetic acid to each well followed by brief gentle agitation.
4. Measure $O.D._{492\ nm}$ using a plate reader. Subtract color control well reading from reaction well reading for each sample. For 30 min incubation, PDH activity in IU/L unit = µmol/(L•min) = O.D. x 1000 x 110 µl /(30 min x 0.6 cm x 18 x 10 µl) = **O.D. x 33.95** (or **8.49** for 120 min incubation)

Additional information:

- Note that "0.6 cm" in the equation is the typical light path in a 96-well plate and may be custom adjusted as needed.
- A 3% Acetic acid solution needs to be prepared for reaction termination.
- The assay solution contains DMSO and iodonitrotetrazolium violet. Please refer to the product page of our website or contact us for MSDS information.