abcam

Product datasheet

Pyruvate dehydrogenase (PDH) Enzyme Activity Dipstick Assay Kit ab109882

27 References 3 Images

Overview

Notes

Product name Pyruvate dehydrogenase (PDH) Enzyme Activity Dipstick Assay Kit

Sample type Cell culture extracts, Tissue

Assay type Sandwich (quantitative)

Species reactivity Reacts with: Mouse, Rat, Cow, Human, Pig

Product overview ab109882 is used to quantify the activity of the PDH enzyme cor

ab109882 is used to quantify the activity of the PDH enzyme complex from human, bovine, mouse, rat and pig samples. This is accomplished by immunocapturing PDH with an anti-PDH antibody immobilized on a defined section of a dipstick. The enzyme complex is immunocaptured in its native form and activity is measured by the reaction scheme. PDH activity is visualized by coupling PDH-dependent production of NADH to the reduction of NBT in the presence of excess diaphorase, forming an insoluble intensely colored precipitate at the capture line. The signal intensity is measured by a dipstick reader or analyzed by other imaging systems such as a flatbed scanner.

Purified mitochondria can be used in this assay. However, homogenized tissue and whole cells can also be used without the need for mitochondrial isolation.

Pyruvate dehydrogenase activity is regulated by PDH kinase and PDH phosphatase. Cells grown in glucose media derive most of their energy from glycolysis; therefore, most of their PDH complex may be in an inactive phosphorylated form at the time of isolation. Growing cells in alternate carbon sources such as glutamine/galactose may up-regulate PDH activity. This kit does not include PDH kinases, PDH phosphatases or their respective inhibitors. These may be incorporated into the assay at the user's discretion.

Store dipsticks at room temperature out of direct sunlight in their provided desiccant container.

Avoid high humidity conditions.

Store 20X Reagent Mix, Detergent, and Diaphorase at -80°C, but these may be aliquoted upon

receipt to prevent freeze/thaw cycles.

Store NBT in the dark at -80°C.

Tested applications Suitable for: Functional Studies

Platform Reagents

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Properties

Storage instructions

Please refer to protocols.

Components	30 tests
20X Reagent Mix	1 x 600µl
96-well microplate	2 units
Blocking solution	1 x 2ml
Detergent	1 x 1ml
Diaphorase	1 x 400µl
Dipsticks	1 x 30 tests
NBT	1 x 40µl
Sample buffer	1 x 30ml

Applications

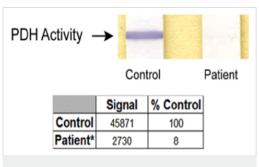
The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab109882 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Functional Studies		Use at an assay dependent dilution.

Images



Pyruvate dehydrogenase (PDH) Enzyme Activity Dipstick Assay Kit (ab109882) Figure 2. An example using the ab109882 to measure PDH activity in human fibroblast sample. Based on the standard curve, 160 μg of protein extract were loaded onto a dipstick for each sample. The figure shows two developed dipsticks, a control sample and a sample from a patient with a mutation in the E1-alpha subunit of PDH. The analysis of the signal intensity and interpolation from the standard curve showed that the patient sample had 8% the PDH activity of the control.

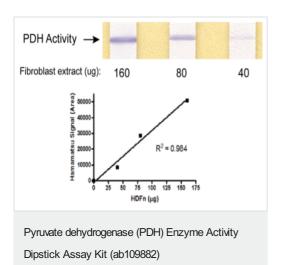
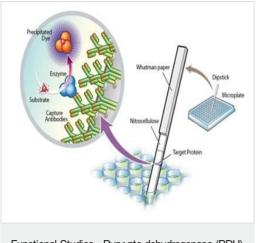


Figure 1. An example using the ab109882 to measure PDH activity in human fibroblast sample. Developed dipsticks from a 1:2 dilution series using a positive control sample and the associated standard curve. Starting material was 160 μg of fibroblast protein extract in a 100 μL reaction volume (50 μL sample in Sample buffer plus 50 μL of Blocking solution). The dipsticks were developed for 1 hour in Activity buffer.



Functional Studies - Pyruvate dehydrogenase (PDH) Enzyme Activity Dipstick Assay Kit (ab109882) Abcam's enzyme activity assays apply a novel approach, whereby target enzymes are first immunocaptured from tissue or cell samples before subsequent functional analysis. Dipstick ELISA Kits extend this concept by utilizing the well-established lateral flow concept, wherein capture antibodies are striped onto nitrocellulose membrane and a wicking pad draws the sample through the antibody bands. All of our ELISA kits utilize highly validated monoclonal antibodies and proprietary buffers, which are able to capture even very large enzyme complexes in their fully-intact, functionally-active states.

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