abcam

Product datasheet

Complex I Enzyme Activity Dipstick Assay Kit ab109720

★★★★★ 1 Abreviews 48 References 4 图像

概述

产品名称 Complex I Enzyme Activity Dipstick Assay试剂盒

样**品**类型 Cell culture supernatant, Cell culture extracts, Tissue

检测类型 Enzyme activity

种属反应性 与反应: Mouse, Rat, Cow, Human

产品概述 Contains 48 dipsticks and necessary components to quantify the activity of the Complex I enzyme

complex in human, bovine, rat and mouse samples. The kit includes sufficient materials to

generate a standard curve and evaluate several unknown samples.

In this assay the specificity of anti-Complex I monoclonal antibodies (mAbs) is combined with the well-characterized Complex I in-gel activity assay that is not rotenone sensitive. First, Complex I is immunocaptured (i.e. immuno-precipitated in active form) on the dipstick. Second, the dipstick is

immersed in Complex I activity buffer solution containing NADH as a substrate and

nitrotetrazolium blue (NBT) as the electron acceptor. Immunocaptured Complex I oxidizes NADH and the resulting H+ reduces NBT to form a blue-purple precipitate at the Complex I antibody line on the dipstick. The signal intensity of this precipitate corresponds to the level of Complex I enzyme activity in the sample. Combined with dipstick assay kit for measuring Complex I quantity (ab109722/MS131 for human sample; ab109875/MS133 for rodent samples), it is possible to

determine the relative specific activity of immunocaptured Complex I. The signal intensity is best measured by a dipstick reader or may be analyzed by a standard imaging system.

经测试应用 适用于: Functional Studies

平台 Reagents

性能

存放说明 Store at +4°C. Please refer to protocols.

组 件	48 tests
10X Blocking Buffer	1 x 400µl
96-well microplate	2 units
Dipsticks	1 x 48 units

组件	48 tests
Extraction Buffer (ab260490)	1 x 15ml
NADH (lyophilized)	1 vial
NBT (Lyophilized) - 10mg	1 vial
Reaction Buffer	1 x 15ml
Wash buffer	1 x 2ml

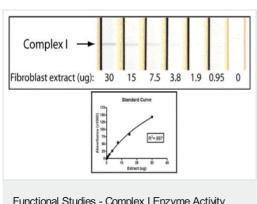
应用

The Abpromise guarantee Abpromise™承诺保证使用ab109720于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

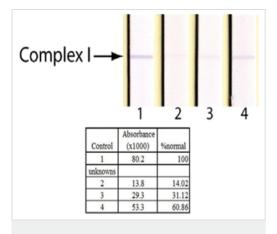
应用	Ab评论	说明
Functional Studies		Use at an assay dependent dilution.

图片

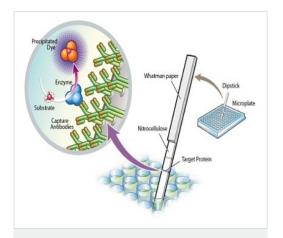


Functional Studies - Complex I Enzyme Activity
Dipstick Assay Kit (ab109720)

An example using ab109720 to measure Complex I activity in human fibroblast samples. Developed dipsticks from a 1:2 dilution series using a positive control sample and the associated standard curve. Starting material was 30 μ g of fibroblast protein extract.

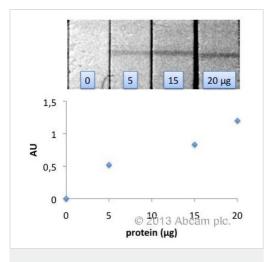


Functional Studies - Complex I Enzyme Activity Dipstick Assay Kit (ab109720) An example using ab109720 to measure Complex I activity in human fibroblast samples. Based on the standard curve, 15 μg of protein extract were loaded onto a dipstick for each sample. The figure shows four developed dipsticks, a control sample (1) and three unknowns (2-4). The analysis of the signal intensity and interpolation from the standard curve showed that the unknown samples have between 14-61% of normal Complex I activity levels.



Functional Studies - Complex I Enzyme Activity
Dipstick Assay Kit - 48 tests (ab109720)

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.



Functional Studies - Complex I Enzyme Activity Dipstick Assay Kit (ab109720)

This image is courtesy of an Abreview submitted by Christian Marx

ab109720 was used to quantify complex I activity in HCT116 cells. The kit was used as described in the manual.

Shortly: cells were scraped and lysed in extraction buffer. Cell fragments were removed bey centrifugation and protein concentration was measured by Bradford assay. Increasing amounts of protein (as shown in image) were applied to each well as standard curve. A standard flatbed scanner was used instead of a Dipstick-reader. Due to the low contrast of the resulting bands, the brightness and contrast of the image was adjusted afterwards. Developed for 45 minutes as recommended by the manual.

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