**Product name**
Complex I Enzyme Activity Dipstick Assay Kit

**Sample type**
Cell culture supernatant, Cell culture extracts, Tissue

**Assay type**
Enzyme activity

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

**Product overview**
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 immuno captures (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 (ab 109722/MS131 for human sample; ab 109875/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.

**Tested applications**
Suitable for: Functional Studies

**Platform**
Reagents

**Properties**

**Storage instructions**
Store at +4°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>48 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Blocking Buffer</td>
<td>1 x 400µl</td>
</tr>
<tr>
<td>96-well microplate</td>
<td>2 units</td>
</tr>
<tr>
<td>Dipsticks</td>
<td>1 x 48 units</td>
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</tbody>
</table>
Components

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction Buffer</td>
<td>1 x 15ml</td>
</tr>
<tr>
<td>NADH (lyophilized) - 2mg</td>
<td>1 vial</td>
</tr>
<tr>
<td>NBT (lyophilized) - 10mg</td>
<td>1 vial</td>
</tr>
<tr>
<td>Reaction Buffer</td>
<td>1 x 15ml</td>
</tr>
<tr>
<td>Wash buffer</td>
<td>1 x 2ml</td>
</tr>
</tbody>
</table>

Applications

Our Abpromise guarantee covers the use of ab109720 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional Studies</td>
<td></td>
<td>Use at an assay dependent dilution.</td>
</tr>
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</table>

Images

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 µg of fibroblast protein extract.
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.

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.
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 by 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.

Please note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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