Cytochrome c Reductase Assay Kit (TBS2116, 100Assays, Store at -20°C)

One-step Sensitive Quantitation for Cytochrome C Reductase Activity in bio-samples

DESCRIPTION

This kit is designed to measure the NADPH cytochrome C reductase activity in biological samples including cell and tissue extracts, and in purified microsomes (endoplasmic reticulum, ER). Eukaryotic NADPH-Cytochrome C reductase (NADPH cytochrome P450 reductase, EC 1.6.2.4) is a flavoprotein localized to the endoplasmic reticulum. It transfers electrons from NADPH to several oxygenases, the most important of which is the cytochrome P450 family of enzymes, responsible for xenobiotic metabolism.

NADPH-cytochrome C reductase is widely used as an endoplasmic reticulum marker and as a biomarker of ecological pollution and dietary lipid uptake. This kit is based on a colorimetric assay that measures the reduction of cytochrome C by NADPH-Cytochrome C reductase in the presence of NADPH. The reduction of cytochrome C results in the formation of distinct bands in the absorption spectrum and the increase in absorbance at 550 nm is measured with time.

APPLICATIONS

- Measuring NADPH-cytochrome c reductase levels in biological samples after exposure to drugs or other xenobiotics.
- Detection of ER during isolation and subcellular fractionation by density gradient separation.

KEY FEATURES

Flexible: The kit can be used for 96-well plate or 384 well plate.
Accurate: Accurate detection of cytochrome C reductase activity in small size as 50 µL samples.
Simple: Just load-Read. Kit can be used as a robust method.
Time saving: Less than 30 minutes.

KIT CONTENTS (100 Assays)

Component	Part Size
Assay Buffer	15 mL
Cytochrome C	1.2 mL
NADPH	2.0 mL
Cytochrome C Reductase Positive	0.25 mL
Cytochrome C Oxidase Inhibitor	1.0 mL

STORAGE AND HANDLING

Store kit at -20°C. Shelf life of 2 years. Protect from light.
Ship with blue ice.

Sample Preparations:

Detergents used to extract biological samples may have an effect on the activity of cytochrome c reductase. CHAPS, sodium cholate, sodium deoxycholate, and TRITON X-100 at concentrations up to 0.05% in the final reaction mixture have been tested with this procedure and were found to be compatible with the assay procedure. When a detergent is used to lyse cells or to extract tissue, it may cause the release of cytochrome c reductase from the ER membrane into the soluble fraction. Centrifugation at 100,000 x g will result in virtually all the activity in the supernatant and very little in the pellet.

PROCEDURES

This assay measures the reduction of cytochrome c by NADPH-cytochrome c reductase in the presence of NADPH. The absorption spectrum of cytochrome c changes with its oxidation/reduction state. Upon reduction a sharp absorption peak is observed at 550 nm. The reduction of cytochrome c is monitored by the increase of cytochrome c absorbance at 550nm. Note that the monitored wavelength is critical, and the deviation must be 2 nm or less. A deviation of 10 nm will result in no signal at all. The assay buffer provided has been determined to be optimal for the enzyme activity. The activity is measured by a spectrophotometer with a kinetic program and is linear for 3-5 minutes when 1-4 milliunits of enzyme are added per assay. The linearity range is 1-6 milliunits for a 1-minute enzyme assay. In crude extracts of tissues or cell lines (1000 x g supernatant), excessive shearing forces during preparation can expose the inner mitochondrial membrane that contains cytochrome c oxidase. This enzyme catalyzes the reverse reaction (oxidation of cytochrome c) under the assay conditions and will cause inaccurate determination of reductase activity. This reaction can be prevented by addition of 20 µL of the Cytochrome c Oxidase Inhibitor Solution to the reaction mixture. This results in a final inhibitor concentration of 1 mM.

Assay Procedures

1. Keep the enzyme preparations (samples and control) on ice. Warm Assay Buffer, Cytochrome C, and NADPH Solution to 25°C before use. Note: Reagent volumes for blank, control, and test samples are summarized in Table 1.
2. Set the spectrophotometer to 550 nm and run the kinetic program at 25°C: Initial delay = 5 seconds Interval= 30 seconds Readings = 7.

For very dilute samples increase the reading time up to 5 minutes.

3. The reaction volume is 200 µL/well.
4. Make a working mixture: make 120 µL Assay Buffer (at 25°C), and 10 µL Cytochrome C Solution for each well. The amount is calculated by assay numbers. Add 130 µL of the mixture to each well.
5. Add 50µL of sample to indicated sample well. If the sample size is less than 50µL, use DD water to bring the volume to 50 µL.
   Note: For samples with expected interference from cytochrome c oxidase activity, add 20 µ: of the Cytochrome c Oxidase Inhibitor Solution.
6. For the positive control reactions, add 25 or 50 µL of Positive Control.
7. Start the reaction by adding 20 µL of NADPH Working Solution. Mix by inversion again.

8. For a blank reaction, measure the value given by the reagents alone without enzyme present.
9. Calculate the activity of the samples assayed as below:

Units/ml= (ΔA550/min x Dil x 0.2) / (Sample Vol x21.1)
ΔA550/min = ΔA(sample) – ΔA (blank)

Dil = dilution factor of sample.
0.2 = reaction volume in ml.
Sample vol = volume of sample or enzyme in ml
21.1=Extinction coefficient (Ɛ mM) for reduced cytochrome C.
Note: In the event the reaction time is longer than 1 minute, divide the A550 by the reaction time to obtain A550/min.

RELATED PRODUCTS

Cell Viability Assay Kits (TBS2001)
CCK-8 Cell Viability (TBS2022)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
Glucose Oxidase Colorimetric/Fluorometric Assay (TBS2088)
Ethanol Colorimetric / Fluorometric Assay (TBS2090)
Tryptase Activity Assay (TBS2101)
β-Hexosaminidase Activity Assay (TBS2105)

Table 1: Reaction scheme

Test	**Assay Buffer**	**Cytochrome C**	**Water**	**Sample or Positive Control**	**NADPH**	**Inhibitor**
Blank	120 µL	10 µL	50 µL	0	20 µL	0
Sample	120 µL	10 µL	50-x µL	X µL	20 µL	0
Sample with interference	120 µL	10 µL	40-X µL	X µL	20 µL	10 µL
Positive Control	120 µL	10 µL	25 µL	25 µL	20 µL	0
Positive Control	120 µL	10 µL	0	50 µL	20 µL	0

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