Free Radical Scavenging Capacity (DPPH) Assay Kit

Catalog No.: abx294424

Size: 96 tests

Detection Range: 17.39 µmol VC/L – 125 µmol VC/L

Sensitivity: 17.39 µmol VC/L

Storage: Store all components at 4°C. Store the Chromogenic Reagent and Standards in the dark.

Application: For detection and quantification of Free radical scavenging capacity in serum, plasma, cell lysates and tissue homogenates.

Introduction

Abbexa's Free radical scavenging capacity Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Free radical scavenging capacity. DPPH is a synthetic organic free radical which can be used to calculate antioxidant activity. The colored compound product of the reaction has an absorbance maximum at 525 nm. The intensity of the color is proportional to the free radical scavenging activity, which can then be calculated by comparing absorbance values before and after addition of sample antioxidants.

Kit components

1. 96-well microplate
2. Chromogenic Reagent: 1 Vial
3. Standard: 2 Vials
4. Brown Plastic Bottle: 1
5. Plate sealer: 2

Materials required but not provided

1. Microplate reader (525 nm)
2. PBS (0.01 M, pH 7.4)
3. Ethanol (anhydrous)
4. Ethanol (80%)
5. Pipette and pipette tips
6. Sterile 2 ml microcentrifuge tubes
7. Ultrasonicator
8. Centrifuge

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum/Plasma:** Samples can be tested directly. Assay immediately or store at -80°C for up to one month.
- **Cell Lysate:** Harvest 1 × 10^6 cells and wash with PBS (0.01 M, pH 7.4). Homogenize in 200 µl of 80 % ethanol by ultrasonication at 4°C, then centrifuge at 10,000 × g for 10 minutes at 4°C. Collect the supernatant and store on ice for immediate detection.
- **Tissue Homogenates:** Carefully weigh 20 mg of tissue and wash with cold PBS (0.01 M, pH 7.4). Homogenize in 180 µl of 80 % ethanol using a Dounce homogenizer at 4°C, then centrifuge at 10,000 × g for 10 minutes at 4°C. Collect the supernatant and store on ice for immediate detection.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with 80 % ethanol, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Horse serum	1
Dog plasma	1
Mouse serum	1
Rat serum	1
10 % Rat liver tissue homogenate	3-6
10 % Rat heart tissue homogenate	1
10 % Mouse heart tissue homogenate	1
10 % Cow liver tissue homogenate	4-8
10 % Mouse lung tissue homogenate	3-6
10 % Mouse stomach tissue homogenate	3-6
1.92 × 10^6 CHO cells lysate	1
2.164 × 10^6 Molt-4 cells lysate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may

lead to erroneous results.

- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Chromogenic Reagent Working Solution**: Dissolve a vial of Chromogenic Reagent with 1 ml of anhydrous ethanol and sonicate for 1 minute to mix thoroughly. Transfer the solution to the provided brown plastic bottle, then add 11 ml of anhydrous ethanol and sonicate for 5 minutes or incubate at room temperature for 2 hours in the dark. The prepared working solution can be stored at 4°C for up to 2 weeks in the dark.
- **12.5 mmol/L Standard Solution:** Dissolve a vial of Standard with 1 ml of anhydrous ethanol and mix thoroughly. The prepared solution can be stored at 4°C for up to 1 week in the dark.
- **125 µmol/L Standard Working Solution:** Mix 10 µl of 12.5 mmol/L Standard Solution with 990 µl of anhydrous ethanol and mix thoroughly. Prepare fresh before use and use immediately.
- **Standard Dilutions:** Prepare standard dilutions as summarized in the following table:

Standard Dilution (µmol/L)	**0**	**25**	**50**	**62.5**	**75**	**87.5**	**100**	**125**
125 µmol/L Standard (µl)	0	40	80	100	120	140	160	200
Anhydrous ethanol (µl)	200	160	120	100	80	60	40	0

For the blank, or 0 µmol/L standard, use pure anhydrous ethanol. The volume of each standard will be 200 µl.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

Serum and Plasma samples

1. Label microcentrifuge tubes for standards, samples, and controls. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 400 µl of standard dilutions to the standard tubes.
3. Add 400 µl of sample to the sample tubes.
4. Add 400 µl of sample to the control tubes.
5. Add 500 µl of Chromogenic Reagent Working Solution to the standard and sample tubes, and 500 µl of anhydrous ethanol to the control tubes.
6. .Mix thoroughly and incubate all tubes at room temperature for 10 minutes in the dark.
7. Centrifuge at 4000 × g for 5 minutes at 4°C, then transfer 180 µl of supernatant from each tube to corresponding microplate wells.
8. Measure the OD of each well with a microplate reader at 525 nm.

Cell Lysate and Tissue Homogenate samples

1. Assign microplate wells for standards, samples, and controls. Each sample requires a corresponding control. It is strongly recommended to prepare all the wells in duplicate.
2. Add 80 µl of standard dilutions to the standard wells.
3. Add 80 µl of sample to the sample wells.
4. Add 80 µl of sample to the control wells.
5. Add 100 µl of Chromogenic Reagent Working Solution to the standard and sample wells, and 100 µl of anhydrous ethanol to the control wells.
6. Mix thoroughly and incubate at room temperature for 10 minutes in the dark.
7. Measure the OD of each well with a microplate reader at 525 nm.

C. Calculation of Results

Plot the standard curve, using the OD of the standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the free radical scavenging capacity in each sample well can be derived with the following formulae:

$$\textbf{Free radical scavenging capacity (µmol VC/L)} = \frac{\boldsymbol{OD_{Blank} - \left(OD_{Sample} - OD_{Control}\right) - b}}{\mathbf{a}} \times \boldsymbol{f}$$

$$\textbf{Free radical scavenging rate } (\%) = \frac{\boldsymbol{OD_{Blank} - (OD_{Sample} - OD_{Control})}}{\boldsymbol{OD_{Blank}}} \times \mathbf{100}\ \%$$

where:

OD_{Blank}	OD value of 0 µmol/L standard blank
OD_{Sample}	OD value of sample
$OD_{Control}$	OD value of control
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
f	Dilution factor of sample before assay