BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Antioxidant Assay Kit (Cat #: A-121)

COMPONENTS: Antioxidant Assay Solution: 20 ml (200 wells), store at 4°C
Ascorbic Acid: 50 mg (positive control), store at 4°C

PRODUCT DESCRIPTION: Naturally occurring antioxidants in many fruits, vegetables, and medicinal plant extracts are known to promote health and delay aging. These compounds prevent free radical formation by interfering with the oxidative process at the initiation, propagation, or termination step. Measurement of the effectiveness of an antioxidant compound therefore represents an important step in formulating strategies aimed at promoting health benefits. The 2,2-diphenyl-1-picrylhydrazyl (DPPH)-based Antioxidant Assay Kit is an improved version of that documented by Shimamura et al (Anal. Sci.30, 717 – 721, 2014), allowing for quick and easy assessment of the antioxidant capacity of a sample in ~30 min. Reaction of DPPH with an antioxidant causes reduced light absorption at 517 nm, which can be used to measure sample antioxidant capacity. The assay kit can be adapted for robust compound screening. Each kit is sufficient for 200 wells.

PROTOCOLS

Preparation of ascorbic acid solution:

First prepare a fresh 10 mM solution by dissolving 2 mg of ascorbic acid in 1.13 ml dH_2O. Dilute the 10 mM solution 10-fold with dH_2O to 1 mM.

Assay:

1. Add 20 µl of dH_2O (or a suitable solvent used for extraction of antioxidants) to a plain (uncoated) 96-well microplate serving as assay blank.

2. Add 20 µl of each properly diluted test sample to the plate. Optional: Add 20 µl of freshly prepared 1mM ascorbic acid as positive control.

3. Reaction is initiated by addition of 100 µl Antioxidant Assay Solution to each well. Mix contents by gentle but thorough agitation for ~10 sec.

4. Cover plate and incubate at 37°C in dark for 30 min. *Do not use CO_2 incubator*.

5. Measure O.D. at 517 nm using a plate reader. The blank well should have the highest absorbance. Presence of antioxidants would reduce the $O.D._{517\ nm}$.

6. $\Delta O.D. = O.D._{Blank} - O.D._{Sample}$. Sample $\Delta O.D.$ is proportional to antioxidant capacity.