

HORAC assay kit

HORAC Assay

CATALOG #

AAA71829

PRODUCT SYNONYMS

HORAC; HORAC Assay; HORAC Assay Kit; HORAC assay kit

PRODUCT SPECIFICATIONS

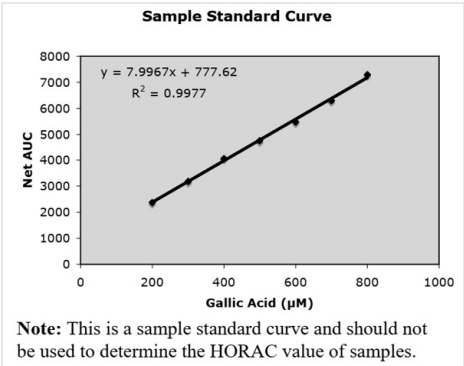
Procedural Notes

1. The entire kit should be stored at 4 degree C until use and all components stored on ice during use.
2. For accuracy, it is recommended that all standards and samples be run in duplicate or triplicate.

Sample Collection and Preparation	All samples should be stored at -70 degree C at all times prior to assay. Apply the samples to the assay plate immediately upon thawing. Sample preparation is subject to the discretion of the user and deviations of the following recommendations may be implemented to optimize or better complement experimental design. Deproteinized Fractions: All of the following samples can be deproteinized and have their nonprotein fractions assayed. To measure the HORAC value in the non-protein fraction, mix the sample with 0.5 M perchloric acid (PCA) (1:1, v/v) or acetone (1:4, v/v), centrifuge at 10,000 x g for 10 minutes at 4 degree C, and recover the supernatant for the assay. Lipophilic Fractions: Antioxidants fall into two classes, hydrophilic and lipophilic, with the later requiring enhancement of solubility in order to measure their capacity in an aqueous environment. One method that can be employed is to dissolve a sample in 100% acetone and then take a portion and dilute it into a solution of 7% -cyclodextrin and 50% acetone. Incubate the sample for 1 hour at room temperature with shaking and then further dilute before running in the assay. Tissue Lysate: Homogenize the tissue sample on ice with ice cold PBS then centrifuge at 10,000 x g for 10 minutes at 4 degree C. Aliquot the supernatant for use in protein determination and running the assay. Cell Culture: Wash the cells 2-3 times with ice cold PBS prior to lysis. Lyse cells by homogenization or sonication with ice cold PBS then centrifuge at 10,000 x g for 10 minutes at 4 degree C. Aliquot the supernatant for use in protein determination and running the assay. Urine: Collected samples may be assayed directly or diluted with Assay Buffer where appropriate. Plasma: Collect the blood with heparin and centrifuge at 4 degree C for 10 minutes. Remove the plasma and aliquot for use in protein determination and running the assay. Blood plasma or serum needs to be diluted 100- to 200-fold with Assay Buffer before it is used in the assay. Nutrition Science: The assay may vary considerably among nutritional substances. Dilution and preparation of nutritional science samples will require foresight and discretion on the part of the user. In many cases, aqueous based samples such as juice and tea can be assayed directly without further processing. Solid food samples or aqueous based samples that contain high amounts of protein and/or fiber may be processed in the following manner: Solid: Weigh the solid and then homogenize with a blender after adding deionized water (e.g., 1:2, w/v). The homogenate is then centrifuged at 12,000 x g for 10 minutes at 4 degree C. The supernatant (water-soluble fraction) is recovered. The water-insoluble fraction (pulp) is washed with deionized water, and the recovered supernatant is pooled with the supernatant obtained from the first centrifugation step. The pooled supernatant is used directly for the assay after suitable dilution with Assay Buffer. The pulp is then further extracted by using pure acetone [1:4, w(pulp)/v] with shaking at room temperature for 30 minutes. The acetone extract is recovered after centrifugation at 12,000 x g for 10 minutes at 4 degree C and is used for the assay after suitable dilution with Assay Buffer. The HORAC activity of the sample is then calculated by adding the HORAC activity from its water-soluble fraction and its acetone-extracted pulp fraction. Aqueous: Centrifuge at 5,000 x g for 10 minutes at 4 degree C to remove particulates then dilute supernatant as needed to run in the assay.
Reagent Preparation	1. The Fluorescein Solution is provided at a 100x concentration and must be diluted with Assay Buffer prior to being used in the assay. To dilute to the working concentration, add 200 µL of the Fluorescein Solution to 19.8 mL of Assay Buffer, this should be labeled as the Fluorescein Working Solution. Store at 4 degree C in the dark for up to one month.

Standard Preparation	In order to create a standard curve for the assay, the stock solution of the Standard must be diluted with the Assay Buffer according to the layout in the table below. Once each standard tube has been created and mixed, 20 µL of each can be added to the appropriate wells on the plate.
	Preparation of Standard Curve.
	Standard
	Tube
	Final Concentration (µM)
	Assay Buffer (µL)
	5mM Stock (µL)
	B0 0 1000 0
	S1 200 960 40
	S2 300 940 60
	S3 400 920 80
	S4 500 900 100
	S5 600 880 120
	S6 700 860 140
	S7 800 840 160.
Preparation and Storage	4 degree C

IMAGES



Standard Curve (Sample)

ADDITIONAL INFORMATION

Related Product Information for HORAC assay kit	Background: It is now well established that oxidative stress is a major risk factor for the development of several diseases including atherosclerosis, cardiovascular disease, and cancer. Oxidative stress is the condition in which there is an imbalance between the concentrations of reactive oxygen species (ROS) and physiological antioxidants, resulting in oxidative damage to many biomolecules within the cell. Products of ROS-mediated oxidation are widely used to monitor oxidative stress. However, it is also important to assess the antioxidant capacity of cells and biological fluids, as well as putative "functional foods" to assess their antioxidant capacity. Organisms possess multiple antioxidant systems to help regulate ROS and prevent oxidative stress. In vertebrates, these include enzymes that metabolize ROS, antioxidant proteins, and smaller molecules that are important antioxidants. These antioxidants include hydrophilic as well as lipid-soluble molecules that are localized throughout various tissues and cell types.
	Principle of the Assay: The HORAC assay is based on the oxidation of fluorescein by hydroxyl radicals via a classic hydrogen atom transfer (HAT) mechanism. Free radicals are generated by hydrogen peroxide (H2O2). The hydroxyl radicals thus generated quench the fluorescence of fluorescein over time. The antioxidants block the hydroxyl radical mediated oxidation of fluorescein until all of the antioxidant activity in the sample is exhausted, after which the H2O2 radicals react with and quench the fluorescence of fluorescein. The area under the fluorescence decay curve (AUC) is used to quantify the total hydroxyl radical antioxidant activity in a sample and is compared to a standard curve obtained using various concentrations of gallic acid. Unlike other antioxidant activity assays, the fluorescent HORAC assay provides a direct measurement of antioxidant capacity against hydrophilic chain-breaking hydroxyl radicals.

References

1. Ou, B., Hampsch-Wodtill, M., Flanagan, J., Deemer, EK., Prior, RL., Huang, D. J Agric Food Chem. 50, 2772 (2002).

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://stage.aaabiotech.com/assay-kits/horac/71829>