Version 1 Last updated 12 December 2018

ab242299
HORAC Assay Kit

View HORAC Assay Kit datasheet:
www.abcam.com/ab242299

For the quantitative measurement of HORAC in cell lysate, plasma, serum, tissue homogenates, and food extracts.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

HORAC Assay Kit (ab242299) is a fast and reliable kit for the direct measurement of HORAC antioxidant capacity.

Each kit provides sufficient reagents to perform up to 192 assays or 48 assays, including blanks, antioxidant standards and unknown samples. The assay is designed for use in single plate microplate readers as well as readers with high-throughput capabilities.

2. Protocol Summary

Prepare standards and samples and add to 96-well plate.

Add Fluorescein Solution to each well and incubate fro 30 mins at room temperature.

Add Hydroxyl Radical Initiator to each well, then immediately add Fenton Reagent to each well and shake for 15 seconds.

Immediately begin reading the plate with a fluorescent microplate reader at 480/530 nm.

Read the plates in increments of 1–5 mins for a total of 60 mins.

Calculate results.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity		Storage condition
	192 tests	48 tests	
96-well Microtiter Plate	2 x 96 well plate	1 x 96 well plate	+4°C
HORAC Fluorescein Probe (100X)	500 µL	75 µL	-20°C
Hydroxyl Radical Initiator (5X)	100 µL	200 µL	+4°C
Gallic Acid Antioxidant Standard	1.5 mL	1.5 mL	-20°C
Fenton Reagent	5 mL	1 mL	+4°C
Assay Diluent (2X)	50 mL	10 mL	+4°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Sample extracts for testing
- Fluorescence microplate reader equipped with a 485 nm excitation filter and 530 nm emission filter

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 Assay Diluent:

6.1.1 Dilute the Assay Diluent 1:2 with deionized water. Mix to homogeneity. Use this for all sample and standard dilutions. Store the 1X Assay Diluent at 4°C.

6.2 Fluorescent Probe:

6.2.1 Dilute the Fluorescein Probe 1:100 with Assay Diluent. Mix to homogeneity. Label this as 1X Fluorescein Solution. Use only enough Fluorescein Probe as necessary for immediate applications.

6.3 Hydroxyl Radical Initiator:

6.3.1 Dilute the Hydroxyl Radical Initiator 1:5 with deionized or distilled water. Mix to homogeneity. Label this solution as 1X Hydroxyl Radical Initiator Solution. Use only enough Hydroxyl Radical Initiator as necessary for immediate use.

Δ Note: Do not store diluted Fluorescent Probe or Hydroxyl Radical Initiator solutions.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Dilute the 5 mM Gallic Acid Antioxidant Standard stock solution in 1X Assay Diluent. Use only enough Gallic Acid Antioxidant Standard as necessary for immediate applications.

7.2 Prepare a series of the remaining antioxidant standards as shown below:

Standard #	Gallic Acid Antioxidant Standard (µL)	Assay Diluent (µL)	Gallic Acid Conc (µM)
1	90	410	900
2	80	420	800
3	70	430	700
4	60	440	600
5	50	450	500
6	40	460	400
7	30	470	300
8	20	480	200
9	10	490	100
10	0	500	0

8. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

8.1 **Deproteinated Fractions:** Samples can be deproteinated and have their non-protein fractions assayed. Mix samples with 0.5 M perchloric acid (1:2, v/v), centrifuge at 10,000 *x g* for 10 minutes at 4°C. Remove the supernatant for measuring the non-protein fraction in the assay.

8.2 **Cell Culture:** Wash cells 3 times with cold PBS prior to lysis. Lyse cells with sonication or homogenation in cold PBS and centrifuge at 10,000 *x g* for 10 minutes at 4°C. Aliquot and store the supernatant for use in the assay.

8.3 **Lipophilic Fractions:** Lipophilic samples must be treated in order to be soluble in an aqueous environment. Dissolve samples in 100% acetone and then dilute in a solution of 7% beta-cyclodextrin and 50% acetone. Incubate the mixture for 1 hour at room temperature with mixing. Further dilute samples as necessary prior to testing.

8.4 **Plasma:** Collect blood with heparin and centrifuge at 4°C for 10 minutes. Remove the plasma and aliquot samples for testing. Blood plasma or serum should be diluted 100-fold or more with Assay Diluent prior to performing the assay.

8.5 **Tissue Lysate:** Sonicate or homogenize tissue sample on cold PBS and centrifuge at 10,000 *x g* for 10 minutes at 4°C. Aliquot and store the supernatant for use in the assay.

8.6 **Plasma or Serum:** Once samples are collected they may be tested after diluted 100-fold with Assay Diluent.

8.7 **Urine:** Once samples are collected they may be tested directly or diluted with Assay Diluent if appropriate.

8.8 **Nutrition Samples:** Nutritional sample results may vary depending on sample source and purification. Dilution and preparation of these samples is at the discretion of the user. Aqueous samples, such as juice extracts, may be tested directly without further manipulation. Solid food samples or high protein samples may be processed as follows:

Solid Samples: Weigh solid sample and then homogenize after adding deionized water (1:2, w/v). Centrifuge the homogenate at 10-12,000 *x g* for 10 minutes at 4°C. Recover the supernatant which is the water-soluble fraction. The solid pulp, or insoluble fraction is also recovered and washed with deionized water. Combine this wash with the supernatant. The pooled supernatant can be diluted with Assay Diluent and used directly in the assay. The pulp is further extracted by adding pure acetone (1:4, w(solid pulp)/v) and mixing at room temperature for 30-60 minutes. Centrifuge the extract/solid at 12,000 *x g* for 10 minutes at 4°C. Recover the acetone extract and dilute with Assay Diluent as necessary prior to running the assay. The total ORAC value is calculated by combining the results from the water-soluble fraction and the acetone extract from the pulp fraction.
Aqueous Samples: Centrifuge the sample at 5-10,000 *x g* for 10 minutes at 4°C to remove any particulates. Dilute the supernatant as necessary prior to running the assay.

Δ Note: Samples should be prepared at the discretion of the user.

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

9.1 Add 20 µL of the diluted Antioxidant Standards or samples to be tested to the 96-well Microtiter Plate.

9.2 Add 140 µL of the 1X Fluorescein Solution to each well. Mix thoroughly. Incubate the plate for 30 minutes at room temperature.

9.3 Add 20 µL of the 1X Hydroxyl Radical Initiator into each well using either a multichannel pipette or a plate reader liquid handling system.

9.4 Immediately add 20 µL of the Fenton Reagent to each well. Shake the plate immediately for 15 seconds to ensure homogeneity.

9.5 Immediately begin reading sample and standard wells with a fluorescent microplate reader with an excitation wavelength of 480nm and an emission wavelength of 530nm. Read the wells in increments between 1 and 5 minutes for a total of 60 minutes. Save values for Calculation of Results below.

Δ Note: The final assay values of blank control should be less than 10% of the initial values in order for the assay to be completed.

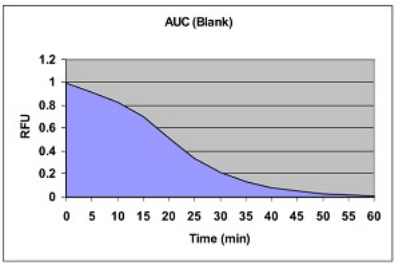
10. Data Analysis

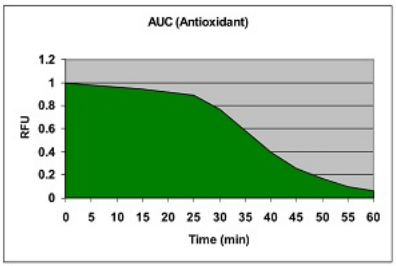
Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

10.1 Calculate the area under the curve (AUC) for each sample and standard using the final assay values and the linear regression formula below. The AUC can be calculated from the equation below:

AUC = 1 + RFU1/RFU0 + RFU2/RFU0 +……+ RFU59/RFU0 + RFU60/RFU0

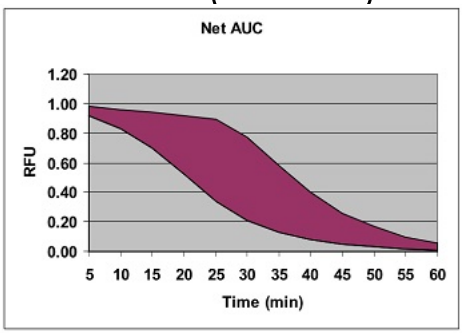
RFU0 = relative fluorescence value of time point zero.
RFUx = relative fluorescence value of time points (e.g. RFU5 is relative fluorescence value at minute five).





10.2 Calculate the Net AUC by subtracting the Blank AUC from the AUC of each sample and standard.

Net AUC = AUC (Antioxidant) – AUC (blank).



10.3 Graph the Net AUC on the y-axis against the Gallic Acid Antioxidant Standard concentration on the x-axis (see Figure 1).

10.4 Calculate the µMole Gallic Acid Equivalents (GAE) of unknown sample by comparing the standard curve. Results (HORAC value) may be expressed as GAE per L or g of sample.

Calculation Example:

20 µL of 10-fold diluted sample is assayed along with 20 µL of each Gallic Acid antioxidant standard including blank as described in Assay Protocol.

The average AUC is 2.2 for blank and 7.0 for sample.
Net AUC = AUC (Antioxidant) – AUC (blank) = 7.0 – 2.2 = 4.8

Based on the Gallic Acid antioxidant standard curve, the equivalent Gallic Acid concentration is 400 µM, therefore,
HORAC value (Sample) = 400 µM x 10 (dilution factor) = 4000 µM
GAE = 4000 µMole GAE/L.

11. Typical Data

Typical standard curve - data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

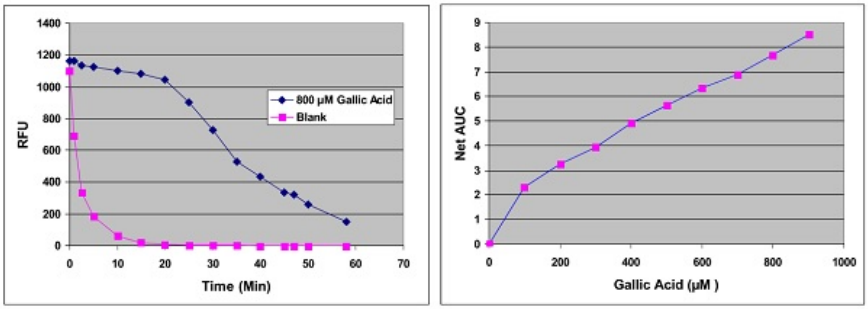


Figure 1. Typical Standard Curve: This standard curve is for demonstration only. A standard curve must be run with each assay.

12. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com 400 921 0189 / +86 21 2070 0500

Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829