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ab233473
ORAC Assay Kit

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

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

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1. Overview

ORAC Assay Kit (ab233473) is a fast and reliable kit for the direct measurement of ORAC antioxidant capacity from cell lysate, plasma, serum, tissue homogenates, and food extracts. Each kit provides sufficient reagents to perform up to 192 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.

Prepare reagents and samples.

Prepare antioxidant standard curve.

Add 1X Fluroscein Solution to each well containing standard or sample and mix well. Incubate for 30 minutes at 37°C

Add Free Radical Initiator Solution to each well and mix thoroughly. Read immediately with a fluorescent microplate reader at Ex/Em = 480/520 nm. Read the wells in increments between 1 and 5 minutes for a total of 60 minutes.

2. Materials Supplied and Storage

Store kit Fluorescein Probe (100X) and Antioxidant Standard (Trolox) at -20°C immediately on receipt. Store all remaining components at 4°C. 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 temperature (before prep)
96-well Microtiter Plate	2	4°C
Fluorescein Probe (100X)	0.5 mL	-20°C
Free Radical Initiator	0.5 g	4°C
Antioxidant Standard (Trolox)	100 µL	-20°C
Assay Diluent (4X)	50 mL	4°C

3. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 480/520 nm.
- 50% Acetone.
- 1X PBS.
- Deionized water.

4. 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.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 1X Assay Diluent

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

5.2 1X Fluorescein Probe

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

Δ Note Do not store diluted Fluorescein Probe solutions.

5.3 Free Radical Initiator

Freshly prepare 80 mg/mL Free Radical Initiator Solution in 1X PBS. For example, weigh out 160 mg of Free Radical Initiator powder in a conical tube and reconstitute the powder with 2 mL of 1X PBS and mix to homogeneity.

Δ Note Free Radical Initiator Solution is not stable and should be used immediately.

5.4 Antioxidant Standard (Trolox)

Ready to use as supplied.

5.5 96-well Microtiter Plate

Ready to use as supplied.

6. Standard Preparation

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

1. Prepare fresh standards by diluting the 5 mM Antioxidant Standard (Trolox) stock solution to 0.2 mM in Assay Diluent (for hydrophilic samples) or 50% acetone (for lipophilic samples) (example: add 10 µL of Antioxidant Standard (Trolox) stock tube to 240 µL of Assay Diluent/50% Acetone).
2. Prepare a series of the remaining antioxidant standards according to the table below.

Standard #	0.2mM Antioxidant Standard (Trolox)	Assay Diluent / 50% Acetone (µL)	Resulting Trolox concentration (µM)
1	50	150	50
2	40	160	40
3	30	170	30
4	20	180	20
5	10	190	10
6	5	195	5
7	2.5	197.5	2.5
8	0	200	0

Δ Note Do not store diluted Antioxidant Standard solutions.

7. 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. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware, however, that this might affect the stability of your samples and the readings can be lower than expected. Avoid multiple freeze-thaws.

7.1 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.

7.2 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.

7.3 Lipophilic Fractions:

Dissolve lipophilic samples in 100% acetone and then dilute in 50% acetone. Incubate the mixture for 1 hour at room temperature with mixing. Further dilute samples as necessary prior to testing.

7.4 Plasma or Serum:

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.

7.5 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.

7.6 Urine:

Test neat or diluted with Assay Diluent if appropriate.

7.7 Solid or High protein Nutrition 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. Separately recover the insoluble fraction (pulp) and wash 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.

7.8 Aqueous Nutrition Sample:

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. Certain liquids such as juice extracts may be tested without dilution.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate or triplicate.

1. Add 25 µL of the diluted Antioxidant Standard or samples to the 96-well Microtiter Plate.
2. Add 150 µL of the 1X Fluorescein Solution to each well. Mix thoroughly. Incubate the plate for 30 minutes at 37°C.
3. Add 25 µL of the Free Radical Initiator Solution into each well using either a multichannel pipette or a plate reader liquid handling system.
4. Mix the reaction mixture thoroughly by pipetting to ensure homogeneity.
5. Immediately begin reading sample and standard wells with a fluorescent microplate reader at 37°C at Ex/Em = 480/520 nm. Read the wells in increments between 1 and 5 minutes for a total of 60 minutes.

9. Data Analysis

1. Calculate the area under the curve (AUC) for each sample and standard using the final assay values and the linear regression:

$$\text{AUC} = 1 + \frac{\text{RFU}_1}{\text{RFU}_0} + \frac{\text{RFU}_2}{\text{RFU}_0} + \frac{\text{RFU}_3}{\text{RFU}_0} + \ldots + \frac{\text{RFU}_{59}}{\text{RFU}_0} + \frac{\text{RFU}_{60}}{\text{RFU}_0}$$

Where:

RFU_0 = relative fluorescence value of time point zero.

RFU_x = relative fluorescence value of time points (e.g. RFU_5 is relative fluorescence value at minute five).

2. Calculate the net AUC by subtracting the Blank AUC from the AUC of each sample and standard

$$Net\ AUC = AUC(Antioxidant) - AUC(Blank)$$

3. Graph the Net AUC on the y-axis against the Trolox Antioxidant Standard concentration on the x-axis (see figure 1 in section 11).
4. Calculate the µM Trolox Equivalents (TE) of unknown sample by comparing the standard curve. Results (ORAC) may be expressed as TE per L or g of sample.

10. FAQs / Troubleshooting

General troubleshooting points are found at
www.abcam.com/assaykitguidelines

11. Typical Data

Data provided **for demonstration purposes only.**

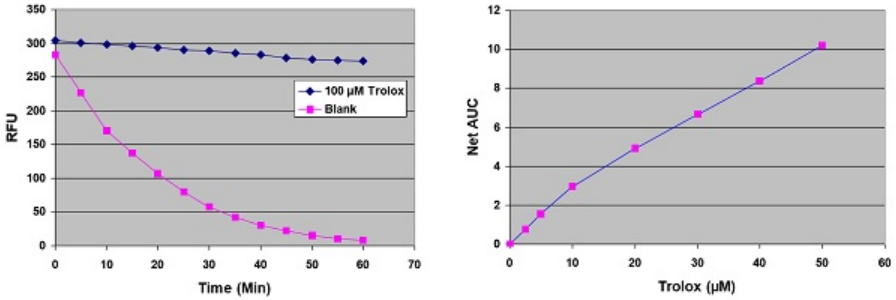

Figure 1. ORAC Activity Assay Standard Curve

12. Notes

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