Product Manual

OxiSelect™ Flavonoid Assay Kit

Catalog Number

XAN-5077 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Flavonoids are an array of polyphenolic compounds containing a benzo-γ-pyrone derivative that have shown to be involved in a number of pharmacological reactions. More than 5000 naturally occurring flavonoids have been found in plants, which are increasingly being researched due to their antioxidant, antibacterial, anti-inflammatory, and antiviral capabilities. Flavonoids are categorized into subgroups according to variations in their heterocyclic chemical structure. These predominant subgroups include flavonols, flavanols, flavanones, flavones, isoflavones, and anthocyanins. They share the base structure that incorporates two benzene rings connected by a heterocyclic pyran ring, which are labeled as A, B, and C below (Figure 1).

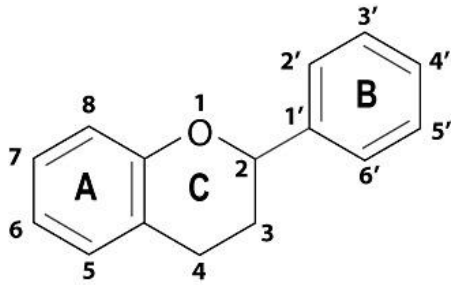

Figure 1. Basic flavonoid structure with two benzene rings (A and B) linked via a heterocyclic pyrane ring (C).

The degree of variation between flavonoids is influenced by the pattern and level of prenylation, glycosylation, hydroxylation, and methylation. Ring substitution positions determine the chemical activity and variability between flavonoids. The substitution, configuration, and number of hydroxyl groups will influence the antioxidant activity of metal scavenging and chelation (Figure 2). Many flavonoids are hydroxylated at the 3, 5, 7, 2, 3',4', and 5' positions. Flavonoid activity and bioavailability are structure-dependent, and although they may exist in their aglycone free unbound state, they predominantly exist as 3-O-glycosides, methylated derivatives, and polymers. Glycosidic linkages usually occur at the 3 or 7 position. While aglycans can be absorbed intestinally, glycosidic forms must be converted to algycans first.

Flavonoids act by diminishing Reactive Oxygen Species (ROS) through metal scavenging or enzyme inhibition, chelating trace elements that generate free radicals, and upregulating antioxidant defense systems. The established anti-inflammatory, antiviral, anticancer, hepatoprotective characteristics of flavonoids are creating new areas of research and drug development. Further research is needed in flavonoid structure and function relationships in order to understand and utilize their therapeutic characteristics.

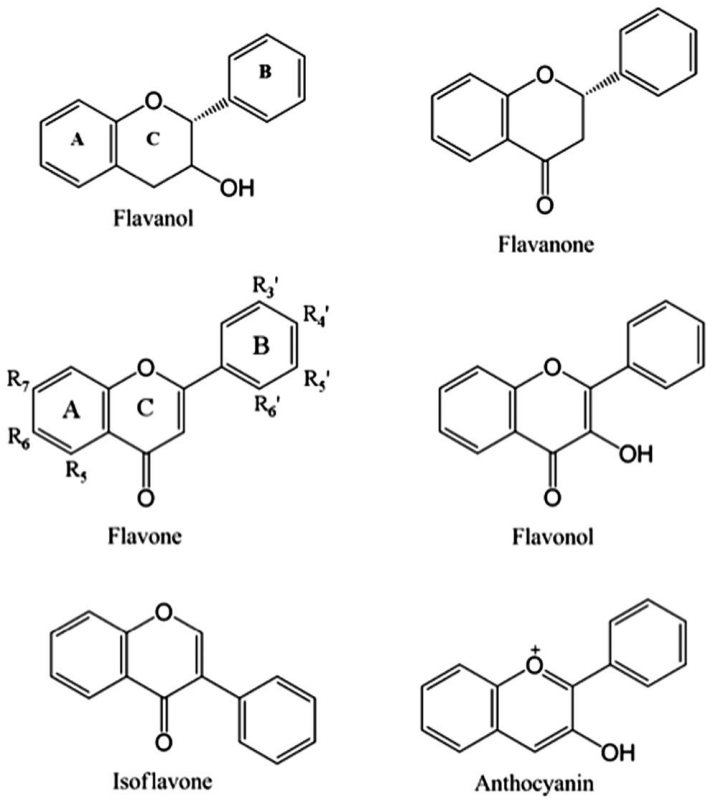

Figure 2. Classes of Flavonoids. The common "R" group substitution positions are highlighted on the Flavone structure.

Cell Biolabs' OxiSelect™ Flavonoid Assay Kit is a quantitative assay for measuring the flavonoid content within various samples such as plasma, urine, plant tissue homogenates, cell extracts, and purified food or drug extracts. The assay can be used for assessing purified or mixtures of flavonoids. The reaction is driven by the scavenging and chelating power of the basic flavonoid structure as well as the hydroxyl and ketone groups that define it. The kit employs quercetin as a standard, which allows the user to determine the flavonoid content present within their sample and express it as Quercetin Equivalents (QE). Quercetin is an ubiquitous flavonoid found in nature from the flavonol subclass category. Each kit provides sufficient reagents to perform up to 200 assays, including standard curve and unknown samples. Due to the variability of flavonoids within samples, the assay may be measured at different wavelengths to determine flavonoid content. The OxiSelect™ Flavonoid Assay Kit offers a simple and efficient analytical method for assessing flavonoid content of a sample in a microplate format.

Assay Principle

The OxiSelect™ Flavonoid Assay Kit is a quantitative assay for measuring the flavonoid content within a sample. A priming solution reacts with the flavonoid ring to enhance the reactivity and detection of the flavonoid. Next, a solution of aluminum ions (Al^{3+}) is added, which complexes with the hydroxyl and keto groups of the flavonoid structure. Finally a hydroxide group stabilizes the ring chemistry and enhances color development. Color development may occur at all stages of the reaction due to the nature of the flavonoids in the sample. After incubating for a few minutes, the sample absorbance is measured at 405-450 nm. Samples can be compared to the quercetin standard for determining flavonoid content. This assay is analytically sensitive to approximately ~2 µg/mL of Quercetin Equivalents.

Related Products

1. STA-312: OxiSelect™ Total Glutathione Assay Kit
2. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay
3. STA-341: OxiSelect™ Catalase Activity Assay Kit
4. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
5. STA-345: OxiSelect™ ORAC Activity Assay
6. STA-347: OxiSelect™ *In Vitro* ROS/RNS Assay Kit (Green Fluorescence)
7. STA-360: OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit
8. STA-802: OxiSelect™ *In Vitro* Nitric Oxide (Nitrite / Nitrate) Assay kit
9. STA-803: OxiSelect™ Myeloperoxidase Activity Assay Kit (Colorimetric)
10. STA-812: OxiSelect™ Glutathione Reductase Assay Kit
11. STA-860: OxiSelect™ Ascorbic Acid Assay Kit (FRASC) (Colorimetric)
12. XAN-5040: OxiSelect™ Trolox Equivalent Antioxidant Capacity (TEAC) Assay Kit (ABTS)

Kit Components **(shipped at room temperature)**

1. Priming Solution (Part No. 50771A): One 12 mL bottle
2. Aluminum Complexing Reagent (Part No. 50772A): One 5 mL bottle
3. Hydroxide Solution (Part No. 50773A): One 20 mL bottle
4. Quercetin Standard (Part No. 50774C): One 200 µL amber tube of 15 mg/mL in DMSO

Materials Not Supplied

1. 96-well microtiter plates
2. 10 kDa MWCO centrifugal filter (for high protein content samples)
3. Ethanol, methanol, DMSO or other organic solvent for sample preparation and extraction
4. Reagents and equipment needed to prepare samples properly
5. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
6. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
7. Conical tubes, microcentrifuge tubes, and bottles for sample and buffer preparation
8. Centrifuge and/or microfuge, rotary vacuum, and other equipment needed for extraction
9. Sonicator, ultrasonic bath, or tissue homogenizer
10. Multichannel micropipette reservoirs
11. Microplate reader capable of reading absorbance between 405-450 nm

Storage

Upon receipt, store the Quercetin Standard at -20ºC and the remaining kit components at room temperature.

Preparation of Samples

These preparation protocols are intended as a guide for preparing unknown flavonoid samples. The user may need to adjust the sample treatment accordingly. All samples should be assayed immediately or stored for up to 2 months at -80°C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. High levels of interfering substances may cause variations in results. A high concentration of protein may interfere with the assay. In this case, filter the sample with a MWCO centrifugal filter before assaying (to reduce protein interference and turbidity). Samples may be diluted as necessary before testing. Run proper controls. Always run a standard curve with samples. Prepare standards in the same solvent as the extracts.

Flavonoid extraction procedures can vary based on the flavonoid structure and the source of sample material. Extraction methods as well as parameters can affect yield. Examples of common methods include Soxhletion, maceration, supercritical fluid, subcritical water, and ultrasonication. Parameters that have been shown to impact flavonoid yield include: (1) extraction solvent, (2) solvent concentration, (3) extraction time, (4) pH, (5) liquid to solid sample ratio, and (6) extraction temperature.

- Plasma: Collect blood sample and add to a blood collection tube containing heparin as the anticoagulant. Centrifuge at 3,000 rpm for 10-15 minutes at 4ºC. Remove the upper yellow plasma supernatant layer without disturbing the white buffy coat (leukocytes). Hydrolyze flavonoid conjugates as necessary. Plasma can be prepared by mixing sample directly with 4 volumes of methanol and incubating for 10 minutes to deproteinize sample. Centrifuge at 12,000 rpm and collect the supernatant. Sample may be tested immediately or rotary vacuumed to evaporate the solvent. Redissolve in 1 mL 0.01% HCl in methanol. Filter to 0.22 µm. Samples should be tested immediately or frozen at -80°C.
- Urine: Hydrolyze flavonoid conjugates as necessary. Urine can be prepared by mixing sample directly with 4 volumes of methanol and incubating for 10 minutes to deproteinize sample. Centrifuge at 12,000 rpm and collect the supernatant. Sample may be tested immediately or rotary vacuumed to evaporate the solvent. Redissolve in 1 mL 0.01% HCl in methanol. Filter to 0.22 µm. Samples should be tested immediately or frozen at -80°C.
- Plant Tissue Extracts: Leaves or tissues may be dried out or freeze died. Mill or grind samples into a fine powder with mortar and pestle. Homogenize/sonicate approximately 1g of tissue in 10-20 mL polar solvent such as ethanol or methanol. Extract by desired method. Centrifuge the homogenate at 12,000 rpm for 5 minutes and collect the supernatant. Filter supernatant through filter paper. Re-extract residue with an equal volume of solvent as desired. Combine supernatants and rotary vacuum dry the filtrate at 40ºC. Store extracts at -80°C protected from light until use. Resuspend in ethanol or methanol prior to testing.
- Food Extracts: Wash uncooked food (e.g. fruits or vegetables) in water and dry out thoroughly or freeze dry. Samples may be milled or ground into a fine powder with mortar and pestle. Samples may also be sonicated in extract solution (e.g. methanol or ethanol) or in an ultrasonic bath. Extract 1-10 g (dry weight) in 1:10 to 1:30 solid to liquid ratio (e.g. 10 g material in 100 mL extraction solution). Allow extraction to proceed at required temperature and time while rotating or refluxing. Vacuum filter the homogenate slurry or centrifuge any remaining debris and collect the extract. Rotary vacuum sample to evaporate the extraction solvent. Store extracts at -80°C protected from light until use. Resuspend in ethanol or methanol prior to testing.

Preparation of Standard Curve

Prepare Quercetin Standards fresh by diluting the stock solution in 75% ethanol (EtOH). The standard may also be prepared in the same solvent used to prepare samples (e.g. methanol or DMSO). Dilute the 15 mg/mL stock to 300 µg/mL in 75% EtOH or other miscible solvent (e.g. add 20 µL of the Quercetin Standard stock solution to 980 µL 75% EtOH). Use this solution to prepare a series of standards according to Table 1 below. Prepare standards immediately prior to each assay performed. Vortex tubes thoroughly. Do not store or reuse the standard preparations.

Note: Some sample solvents may not be compatible with microplate plastics such as polystyrene. Ethanol, Methanol, and DMSO are recommended for preparing and diluting standards and samples.

Standard Tubes	300 µg/mL Quercetin Standard (µL)	Ethanol (µL)	Quercetin (µg/mL)
1	500	500	150
2	500 of tube #1	500	75
3	500 of tube #2	500	37.5
4	500 of tube #3	500	18.8
5	500 of tube #4	500	9.4
6	500 of tube #5	500	4.7
7	500 of tube #6	500	2.3
8	0	500	0

Table 1. Preparation of Quercetin Standards.

Assay Protocol

1. Each standard, sample and control should be assayed in duplicate or triplicate.
2. Add 100 µL of each standard, unknown sample or control to a 96-well plate.
3. Add 50 µL of the Priming Solution to each well and mix thoroughly by pipetting or with a horizontal shaker. Allow the reaction to proceed at room temperature for 10 minutes.
4. Add 25 µL of the Aluminum Complexing Reagent to each well and mix thoroughly by pipetting or with a horizontal shaker. Allow the reaction to proceed at room temperature for 10 minutes.
5. Add 100 µL of the Hydroxide Solution to each well and mix thoroughly by pipetting up and down repeatedly several times or with a horizontal shaker until plate solution appears homogenous. Allow the reaction to proceed at room temperature for 10 minutes on a horizontal shaker.
6. Read the absorbance of each well on a microplate reader using 405-450 nm as the primary wave length.

Note: Sample wells may produce a different color than the quercetin standard based on the type of flavonoids present. The optimal optical density wavelength for reading samples will vary depending on the flavonoids present in the sample.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Calculate the net OD by subtracting the zero standard value from samples and standards. This is the background correction.
3. Graph the standard curve (see Figure 3).
4. Compare the net OD of each sample to the standard curve to determine the quantity of Quercetin Equivalents (QE), as µg/mL equivalents present in a dry extracted sample, according to the equation below. Only use values within the range of the standard curve.
5. Flavonoid Content is expressed as µg of Quercetin Equivalents

FC = Flavonoid Content (µg Quercetin Equivalents per mg of extract)
QE = Quercetin Equivalents concentration in sample extract solution based on standard curve absorbance (µg/mL)
V = Volume of extract (mL)
DF = Dilution Factor
W = Mass of sample used in experiment (mg)

$$FC = \left[\frac{QE \times V \times DF}{W} \right]$$

Example of Results

The following figures demonstrate typical Flavonoid Assay quercetin standard results at 405 nm and 450 nm. One should use the data below for reference only. This data should not be used to interpret actual results.

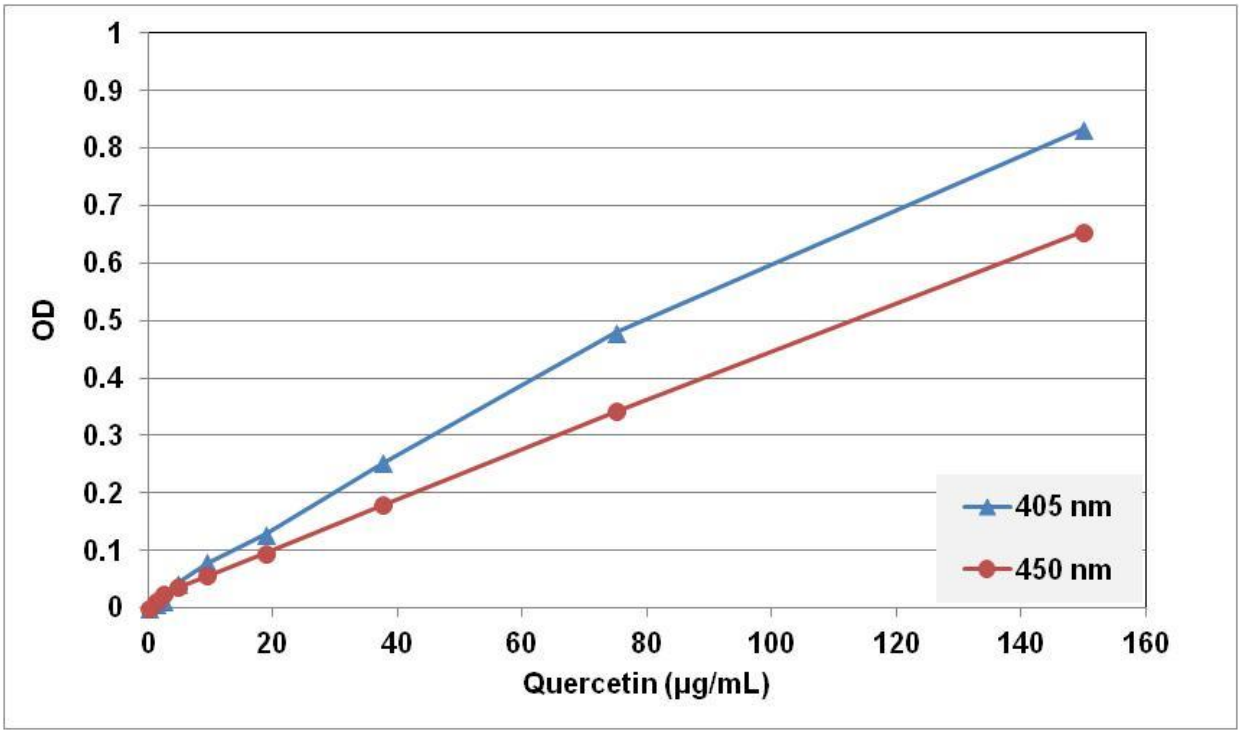

Figure 3. Comparison of Quercetin Standard Curves at 405 nm and 450 nm.

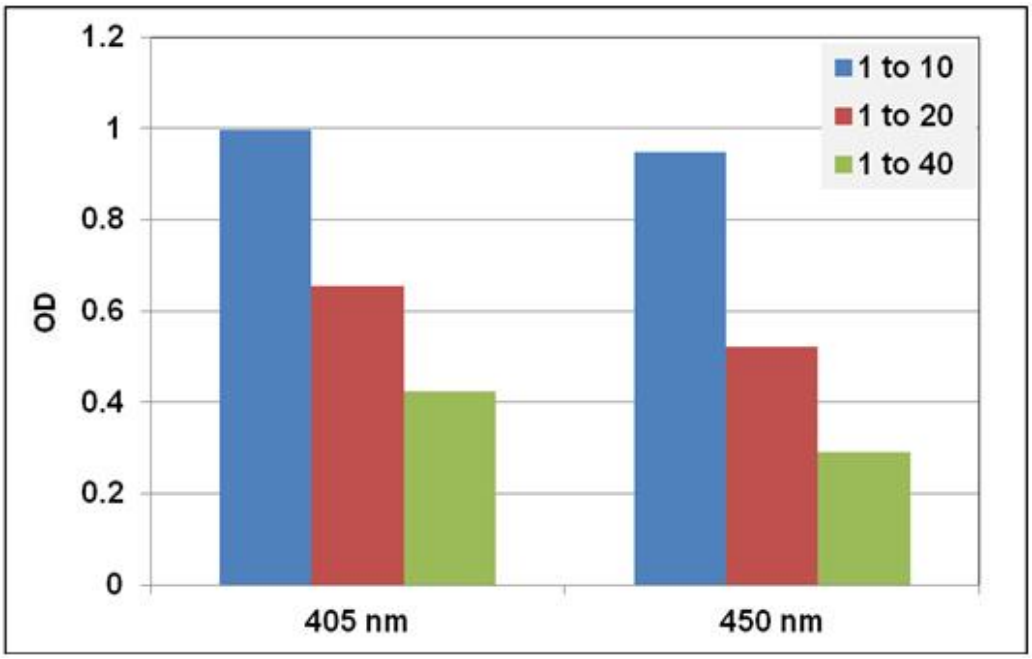

Figure 4. Comparison of blueberry extracts tested with the Flavonoid Assay at 405 nm and 450 nm.

References

1. Bunea, A., et al. *Phytochemistry* (2013) **95**: 436-444.
2. Hollman, P.C.H., et al. *FEBS Lett.* (1997) **418**: 152-156.
3. Vazhappilly, C.G., et al. *J. Nutr. Biochem.* (2017) **45**: 1-14.
4. Weatherby, L., et al. *J. Biol. Chem.* (1943) **48**: 707-709.

Recent Product Citations

1. Olennikov, D. N. (2023). Separation, Characterization and Mammal Pancreatic Lipase Inhibitory Potential of Cucumber Flower Flavonoids. *Separations*. **10**(4):255. doi: 10.3390/separations10040255.
2. Saka, S.O. et al. (2023). Nutrigenomic Effects of White Rice and Brown Rice on the Pathogenesis of Metabolic Disorders in a Fruit Fly Model. *Molecules*. **28**(2):532. doi: 10.3390/molecules28020532.

Warranty

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