Determine the Antioxidant Capacity (per mass) of two Samples of Coffee, fresh blueberries, fresh Kale and the juice of a fresh orange

Using ABEL® antioxidant capacity assays and the ABEL-RAC™ method

METHODS

Description of samples

Table 1

Sample ID	Description
Higham Miller Coffee Ltd – Exhale Coffee	Brown coffee beans
Blueberries	Loose blueberries
Kale	Loose curly kale
Orange juice	Fresh squeezed orange juice

Materials

- Exhale Coffee beans (supplied by Higham Coffee Ltd in a sealed foil lined pouch).
- Hario Skerton plus ceramic coffee mill, supplied by Higham Coffee Ltd, new and unopened which was adjusted at Knight Scientific to one complete turn less than finest grind.
- Bodum Cafetiere (supplied by Higham Coffee Ltd, new and unopened).
- Waring Commercial Blendor Blender (Knight Scientific Ltd).
- Fresh blueberries (Co-op, best before 17/09/2020, tested 16/09/2020).
- Fresh kale (Co-op, best before 20/09/2020, tested 16/09/2020).
- Fresh oranges (Co-op, 25/09/2020, tested 17/09/2020).
- Still bottled spring water (Lidl Simply Still Spring Water)

Protocol for preparation of samples for testing

Exhale Coffee Beans

The packet was opened and 17g of beans was weighed and transferred to the ceramic coffee mill. The mill had been set to one complete turned less than maximum fine. Prior to the experiment, the mill had been commissioned at in our laboratory by having a trial run with another manufacturer's coffee bean. The coffee mill was cleaned by being wiped with white paper towel and still water to remove the remaining coffee grounds. To ensure there was no contamination, before adding the 17g sample of Exhale coffee beans, an additional 5g of the beans were ground through the mill and disposed of. It took 126 turns of the handle to grind the entire 17g of coffee beans. The ground coffee was added to the Bodum cafetiere

which had been commissioned by added three changes of boiling water to it. 275ml of boiled bottled still water (30 seconds after boiling) was added to the cafetiere. After waiting 30 seconds, the coffee was stirred, and left for 4 minutes before depressing the plunger and pouring the coffee liquid from the cafetiere into a 250mL glass beaker. This was in line with the protocol provided by Higham Coffee Ltd.

Blueberries

The blueberries were prepared by weighing 17g which were then added to the jug of a stainless steel commercial blender. 275ml of still bottled water was then added on top of the blueberries. This was homogenized in 2 second bursts for a total of 30 seconds. The homogenized blueberry preparation was poured into 6x50ml tubes, and placed in a Centaur 2 centrifuge at 3000RPM for 5 minutes to separate the insoluble and soluble components of the homogenate. After centrifugation the soluble supernatant was poured off each centrifuge tube and combined in a 250mL conical flask.

Kale

The kale was prepared by weighing out 17g, which was then added to the jug of a stainless steel commercial blender. 275ml of still bottled water was then added on top of the kale. This was homogenized in 2 second bursts for a total of 30 seconds. The homogenized kale preparation was poured into 6x50ml tubes and placed in a Centaur 2 centrifuge at 3000RPM for 5 minutes to separate the insoluble and soluble components of the homogenate. After centrifugation the soluble supernatant was poured off each centrifuge tube and combined in a 250mL conical flask.

Orange

The orange was peeled and the flesh was squeezed by hand into a 50mL tube to collect the fresh orange juice.

Protocol for sample testing

For each sample of blueberry and kale extract, only the soluble components were used. For the orange, the juice was tested directly.

All 4 samples were prepared at a concentration of 10µL/mL with still water by adding 60µL of the sample to 6mL of water in 15mL centrifuge tubes. The 4 samples containing 6mL solutions were gently mixed on a tube rolling machine which is used for gently rolling tubes, set at a fixed speed for 15 minutes to ensure homogeneity and complete solubility. From the 6mL 10uL/mL solutions, a wide range of concentrations were produced: 10uL/mL, 1uL/mL, 100nL/mL, 10nl/mL, 1nL/mL. These were tested in order to identify the concentration range in which the EC_{50} (the concentration that reduced light by half). **See Appendix 1 on background of ABEL-RAC.** Knowledge of this enables us then to produce another set of dilutions

between the highest and lowest point of this range into a narrow range of dilutions from which the EC_{50} is obtained.

A narrow range of concentrations was tested in the ABEL® antioxidant test with singlet oxygen against a no sample control. The coffee sample was tested at narrow range: 100nL/mL, 50nL/mL, 25nL/mL and 12.5nL/mL, the blueberry and kale samples were tested at 5uL/mL, 2.5uL/mL, 1.25uL/mL and 0.625uL/mL, and the orange juice was tested at 1uL/mL, 0.5uL/mL, 0.25uL/mL and 0.125uL/mL. The individual scores were luminescent readings with the lower the light emitted compared to the control the higher the antioxidant capacity of the sample test. The results of these individual reading are used to generate ABEL-RAC mg scores. Details of the method are contained in the background documents and the report sheets for each sample tested are contained in Appendix 2. The whole process was carried out one sample at a time before beginning the preparation of the next samples.

The antioxidant capacity of the sample was evaluated using the ABEL®-RAC method and singlet oxygen as the reactive oxygen species (ROS) challenge in standard ABEL® antioxidant assays with Pholasin®. This is a proprietary method developed by Knight Scientific, in which the sample is mixed with Solution 1, buffer, and Pholasin®; a bioluminescent protein that creates light when in contact with reactive oxygen species and free radicals. During the assay, Solution 2 is injected onto the sample while light is being measured. When solution 2 is added the reactive oxygen species singlet oxygen is generated instantaneously. See Appendix I for more information about singlet oxygen.

RESULTS

ABEL-RAC™ score per µL of Coffee samples in ABEL® singlet oxygen assay		
Sample	**ABEL-RAC Score**	
	Repeat 1	Repeat 2
Higham Miller Coffee Ltd (Exhale Coffee)	7128	7227

ABEL-RAC™ score per µL of fresh produce samples in ABEL® singlet oxygen assay	
Sample	**ABEL-RAC Score**
Blueberries	133
Kale	191
Orange	719

Conclusion and Discussion

The results are expressed as ABEL®-RAC units per mg of samples; the test is carried out in volume measures; 1uL is equivalent to 1mg. As a 2 person cafetiere holds

275mL, it can be presumed a small cup of coffee is approximately 130mL. The mean of the two repeats was 7178. Based on this, it can be said that one small cup of Higham Miller Coffee Ltd Exhale Coffee delivers 933 million ABEL-RAC singlet oxygen antioxidant units (ie 7178 x 1000 x 130 = 9.33 x 10^8). A large 275mL cup would deliver 1.97 billion singlet oxygen antioxidant units.

Comparing the blueberries to the Exhale coffee, one portion of blueberries is 74 grams (sample size 17g), therefore one portion has an ABEL-RAC score of 579 (133 x 4.353 = 579). This equates to 50 blueberries. To get the same amount of antioxidant as 1 cup of Higham Miller Ltd Exhale Coffee made from 17g of coffee beans, you would have to consume 619 blueberries or approximately 916g.

One portion of kale is ~67 grams, therefore one portion has an ABEL-RAC score of 753 (3.94 x 191 = 753). To get the same amount of antioxidant as 1 cup of Higham Miller Ltd Exhale Coffee made from 17g of coffee beans, you would have to consume 643g of kale (approximately 4 large bags).

130mL freshly squeezed orange juce delivers 93 million ABEL-RAC singlet oxygen antioxidant units (ie 719 x 1000 x 130 = 9.35 x 10^7). Therefore, you would need to consume approximately 10 glasses of orange juice of the same size to get the same antioxidant capacity (or power) of a cup of Exhale Coffee. For a small 130mL cup, this equates to 1.3L, which is the juice of approximately 26 oranges (50ml per ~125g medium orange). For a large 275ml glass of orange juice, this equates to 197 million ABEL®-RAC singlet oxygen units, compared to a large 275ml cup of coffee that equates to 1.987 billion. Therefore, to get the same antioxidant ABEL-RAC units as a large cup of Exhale Coffee, you would need to consume approximately 2.75L of fresh orange juice, which would be from approximately 55 oranges.

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APPENDIX 1

TESTING ANTIOXIDANT CAPACITY OF INGREDIENTS AND PRODUCTS
BACKGROUND

ABEL® is an acronym for Analysis By Emitted Light. In the ABEL® antioxidant assays light is generated by a reaction between the photo-protein Pholasin® protein and a variety of reactive oxygen species (ROS). If a material to be tested for potential antioxidant capacity is challenged with one or more of these reactive species in the presence of Pholasin, then any antioxidants in the sample will compete with Pholasin® for these ROS. The result of this competition is a reduction in the amount of light emitted, sometimes referred to as quenching. A light response curve is produced for each sample tested.

The ABEL® (Analysis By Emitted Light) antioxidant test kits, of Knight Scientific Limited, measure the capacity of a sample to scavenge free radicals and other reactive oxygen species (ROS). If the sample has already been exposed to ROS then its antioxidant capacity will have been reduced. Natural materials, such as plants, will have variable antioxidant capacities depending upon conditions under which they were grown as well as the condition of their storage after harvest. Shelf life can also be determined by measuring the loss of antioxidant capacity over time.

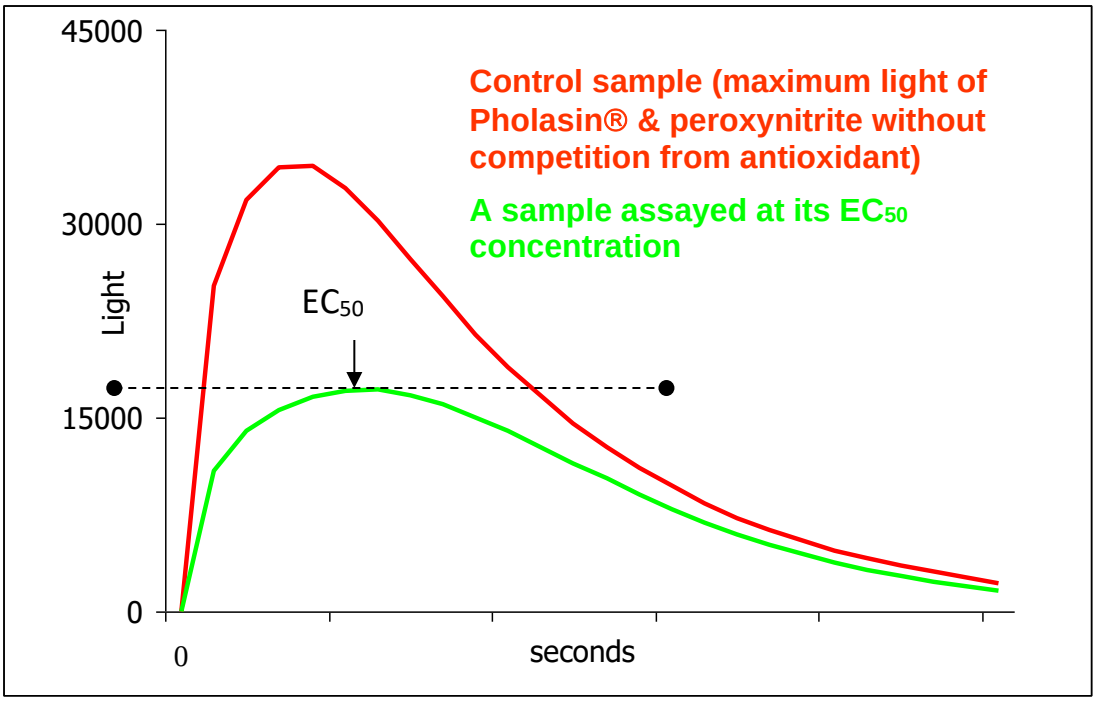
Knight Scientific has developed a highly accurate testing method for quantifying the antioxidant capacity of materials per mg. An ABEL-RAC (relative antioxidant capacity) mg score can be used to adjust the final amounts of ingredients in repeat formulations as well as reject unsuitable ingredients. In this way products based on natural products can be matched batch to batch.

ANTIOXIDANT CAPACITY SCORES: EC_{50} VALUES AND ABEL-RAC mg SCORES

Separate light response curves are produced for each concentration of a material tested as well as the no sample control. The results are presented as EC_{50} values and ABEL-RAC scores. The EC_{50} (effective concentration mg) is the concentration (normalised to g/L or mg/mL) of a material that reduces the light (produced with Pholasin® and the free radical or other reactive oxygen species) by 50%. This reduction in light is the antioxidant capacity of the test material.

The greater the amount of material that is required to reduce the light by half, the weaker the antioxidant capacity. The amount of material required to reduce the light be half is termed the EC_{50} (50% effective concentration). Materials with very high antioxidant capacity have very low EC_{50} values. To make it more readily understandable these EC_{50} values have been converted to positive relative antioxidant capacity scores (ABEL-RAC mg scores) for each free radical or oxidant used to challenge the test material. For examples when peroxynitrite is used as the ROS challenge then the result will be expressed as ABEL-RAC mg Peroxynitrite, for superoxide challenge ABEL-RAC mg superoxide. ABEL-RAC mg scores are the reciprocal of the EC_{50} values multiplied by 100 ($1/EC_{50}$ x 100). The higher ABEL-RAC mg score the higher the antioxidant capacity of the sample.

In the figure below a sample to be tested for antioxidant capacity is challenged with a particular free radical or oxidant in the presence of the light-emitting protein Pholasin®. The concentration that reduced the light by 50% is determined from the results of a range of concentrations and analysed using an exponential regression curve. A template is used to obtain this value. This is the effective concentration (EC_{50}) of the sample. The EC_{50} is converted to an **ABEL-RAC™** score using the formula: **$1/EC_{50}$ (mg) x 100**. ABEL-RAC™ scores, for all the different challenges can be expressed per mg, per dose or percentage in a formula as well as per unit cost.



ABEL-RAC scores are expressed per mg of dried material or µL of a liquid for each of the ROS used to challenge the material. Some materials will be better antioxidants against some free radicals than others.

There are currently seven type-specific antioxidant assays, each using a different kind of free radical or non-radical ROS challenge. These are:

- high concentration superoxide assay (ABEL 20 series),
- halogenated oxidant (= hypochlorous acid) assay (ABEL-30 series)
- peroxynitrite assay (ABEL-40 series)
- hydroxyl radical assay (ABEL 50 series)
- enzyme generated superoxide assay (ABEL 60 series)
- peroxyl radical assay (ABEL-70 series)
- singlet oxygen assay (ABEL-80 series)

TYPE- SPECIFIC ANTIOXIDANT ASSAYS

- **ABEL®-20 series: Antioxidant Test using superoxide** and other free radicals. In this assay a bolus of superoxide is produced in the assay, and is used to challenge the test material. This is an excellent assay for measuring the activity of antioxidants such as ascorbic acid that are chemical quenchers of superoxide. The ABEL®-60 series (see below) is recommended for measuring superoxide dismutase (SOD) activity.
- **ABEL®-30 series: Antioxidant Test using halogenated oxidants** such as hypochlorous acid. Hypochlorous acid is produced by activated white blood cells during inflammation and infection. In this assay hypochlorous acid, derived from chloramine-T, is used to challenge the test material. This assay is excellent for measuring the antioxidant capacity of materials dissolved/dispersed in both aqueous and organic solvents. This assay is very useful in quantifying antioxidant capacity of materials containing phospholipids, fatty acids, sterols and sphingolipids. Hypochlorous acid attacks the double bond in cholesterol as well as primary amine-containing phospholipids which are oxidized to longer lived oxidants such as the chloramines.

- **ABEL®-40 series: Antioxidant Test using peroxynitrite**. In this assay, the peroxynitrite anion ($ONOO^-$) is produced continually in the assay[1].where it is used to challenge the test material. Peroxynitrite is a very reactive oxidant that will attack lipophilic antioxidants such as Vitamin E as well as the hydrophilic antioxidant glutathione. Peroxynitrite attacks virtually any antioxidant or molecule with a double bond irrespective of whether the antioxidant is free in solution or bound to other molecules; it will also penetrate cell membranes. In addition to its use in testing ingredients and products it is also used as a total antioxidant capacity (TAC) test for quantifying antioxidant capacity of the blood of people and animals[i].
- **ABEL®-50 series: Antioxidant Test using hydroxyl radicals** produced in the assay to challenge the test material. The hydroxyl radical is the most reactive free radical known and will attack virtually anything in its path. It should, however, be noted that many organic solvents used to dissolve ingredients used in cosmetic preparations, such as ethanol, methanol and DMSO, are very strong quenchers of hydroxyl radical. While it is possible to control for the antioxidant properties of such solvents, the halogenated oxidant and peroxynitrite assays are recommended as the most appropriate assays for materials dissolved in such solvents.
- **ABEL®-60 series: Antioxidant Test for superoxide, superoxide dismutase and mimetics of SOD**. In this assay superoxide is produced continually at precisely controlled rates and reflects the production of superoxide by activated white blood cells as sites of inflammation as well as from xanthine oxidase. It is sensitive enough to measure the amount of superoxide produced by a small number of living cells as well as for quantifying very low concentrations of SOD. Using this assay the antioxidant capacity of an ingredient or product can be expressed as moles of superoxide that can be quenched or as SOD equivalent units. The assay can distinguish between ingredients or products that are quenchers of superoxide and those that are SOD mimetics.
- **ABEL®-70 series: Antioxidant test using peroxyl radicals**. It measures the specific antioxidant capacity of a sample to quench peroxyl radicals. The results are expressed either in moles of peroxyl radical quenched per minute per mg of test material or more usually as ABEL-RAC units per mg.
- **ABEL®-80 series: Antioxidant test using singlet oxygen.** It measures the capacity of a sample to quench singlet oxygen which is generated in the assay. It is an excellent assay in determining the suitability of materials as antioxidants with the potential to prevent lipid oxidation, resulting from singlet oxygen attack.

  Edible oils can be oxidized during processing and storage via photosensitized oxidation, in which singlet oxygen reacts directly with double bonds. The conjugated and non-conjugated lipid hydroperoxides formed by singlet are decomposed to produce off-flavour compounds with the reduction in quality of the oil. Other factors that affect the oxidative stability of edible oils are temperature, light, processing and fatty acid composition of the oils. Fish oils, for example, are especially suited for antioxidant analysis with this test as they contain significant amounts of polyunsaturated omega-3 fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

  [Molecular oxygen, though a free radical, is chemically inert due to its two unpaired electrons in its outermost orbit having the same quantum number which imposes a spin restriction as electrons spinning in the same direction are unreactive. This restriction can be removed by moving one of the unpaired electrons in a way that alleviates this restriction. This mechanism requires input of energy and generates the singlet states of oxygen: Delta singlet oxygen ($^1\Delta_g\ O_2$) and Sigma singlet oxygen ($^1\Sigma_g\ O_2$) with Delta

[1] Superoxide and nitric oxide are released simultaneously from SIN-1 (3-morpholinosydnonimine hydrochloride) where they react together to produce the peroxynitrite anion ($ONOO^-$).

singlet oxygen the most common in biological systems and usually decays to the Sigma state.]

The scores per mg or µL for each ingredient can be used directly in formulations to determine the theoretical total ABEL-RAC score for the finished product. The theoretical score is then compared to the actual score of the finished product. By determining the ratio of the actual to the theoretical ABEL-RAC score it is possible to quantify positive or negative synergy.

THE TABLE BELOW GIVES A SIMPLE EXAMPLE TO ILLUSTRATE THIS.

INGREDIENTS	ABEL-RAC-mg	PERCENT IN PRODUCT	ABEL-RAC contribution
A	20000	25	5000
B	4000	50	2000
C	7000	20	1400
	SUM		8400
ABEL-RAC	complete	product	14600
	synergy		+1.74

SOME ABEL-RAC peroxynitrite scores determined for particular samples tested

Berberis	6300	Ginger	4000
Bee pollen	9520	Grapeseed extract sample 1	390000
Bilberry	7100	Grapeseed extract sample 2	350000
Broccoli extract	6600	Hawthorn	2600
Cayenne	850	Mint	16000

Curcumin	43000	Picrorhiza	18181
Dandelion	19100	Red sage root	20400
Gallic Acid	122160	Rosemary (second extract)	21500
Gingko leaves	4900	Rosemary (first extract)	819100
Gingko extract	100632	Urtica	30075

These scores are specific to the material actually tested and cannot be used generically for a type of material. Each new batch needs to have its own specific ABEL-RAC mg score and a date of testing.

APPENDIX 2

ABEL-RAC™ using a LINEAR regression of 4 points 17/09/2020

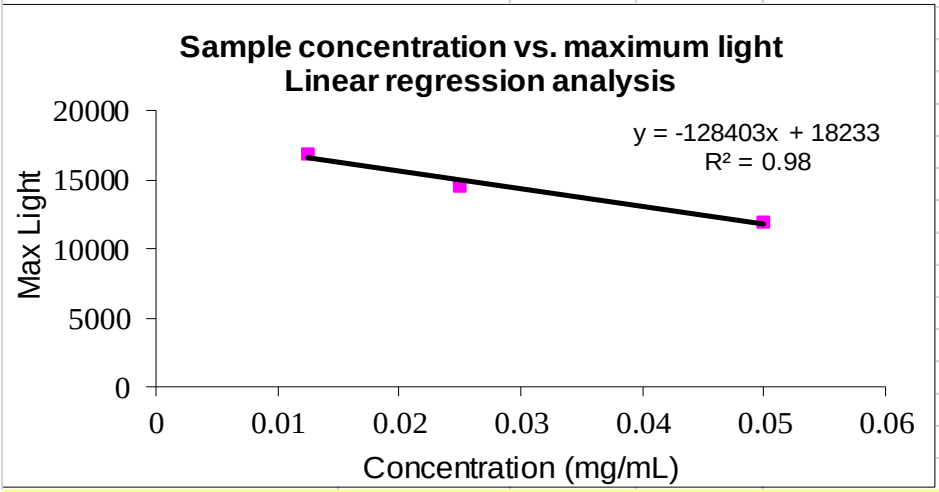
The EC_{50} is the concentration of a material that reduces the light by 50% compared to a solvent control. The EC_{50} value (mg/mL) of a sample is calculated from the equation of a linear regression analysis obtained from plotting the sample concentration (mg/mL) against peak light values taken from the light response curves. ABEL-RAC™ scores are calculated by multiplying the reciprocal of the EC50 value by 100

1. Copy and paste 'Maximum' values and enter sample concentrations (starting concs)

Sample	Maximum Light					
mg/mL	repl1	repl2	mean	% quenching		
Solvent control	18759	18145	18452			
0.1	8747	8585	8666	**53**	1.3	
0.05	11888	12002	11945	**35**	0.7	
0.025	14366	14885	14626	**21**	2.5	
0.0125	16522	17263	16893	**8**	3.1	

2. Graph (plots 'Concentrations mg/mL' vs 'Maximum Light').The trendline, equation and R^2 value are automatically displayed.

The R^2 value must be ≥ 0.99



Intercept	18233
Slope	-128402.857

4. Evaluation template

This evaluation template is used to calculate the final ABEL-RAC™ score per mg of sample.
Input values highlighted in the table below for the sample size and total assay volume (µL)

Assay	singlet oxygen	
Sample ID	Exhale coffee	
Batch No.	Higham Miller Coffee Ltd	
Solvent	water	
Solvent control	18759	18145
Solvent control Mean	18452	
Standard Deviation	434	
%cv	**2.35**	
Y=1/2 Solvent control	9226	
X=EC50	0.07014447	
Sample Volume (uL)	**40**	
Total volume of well (uL)	**200**	
Dilution Factor	5	
EC50 Value	0.0140	mg/mL
ABEL-RAC™	7128	

CV of solvent control must be ≤ 3.0

ABEL-RAC™ using a LINEAR regression of 4 points

17/09/2020

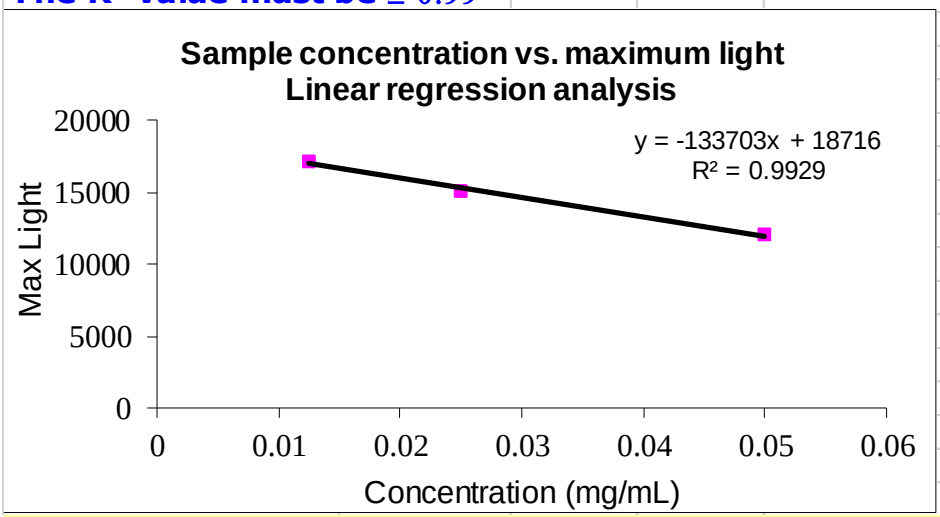
The EC_{50} is the concentration of a material that reduces the light by 50% compared to a solvent control. The EC_{50} value (mg/mL) of a sample is calculated from the equation of a linear regression analysis obtained from plotting the sample concentration (mg/mL) against peak light values taken from the light response curves. ABEL-RAC™ scores are calculated by multiplying the reciprocal of the EC50 value by 100

1. Copy and paste 'Maximum' values and enter sample concentrations (starting concs)

Sample	Maximum Light					
mg/mL	repl1	repl2	mean	% quenching		
Solvent control	18905	18954	18930			
0.1	9454	9551	9503	**50**	0.7	
0.05	12297	11927	12112	**36**	2.2	
0.025	14974	15282	15128	**20**	1.4	
0.0125	17265	17150	17208	**9**	0.5	

2. Graph (plots 'Concentrations mg/mL' vs 'Maximum Light').The trendline, equation and R^2 value are automatically displayed.

The R^2 value must be ≥ 0.99



Intercept	18716
Slope	-133702.857

4. Evaluation template

This evaluation template is used to calculate the final ABEL-RAC™ score per mg of sample.
Input values highlighted in the table below for the sample size and total assay volume (µL)

Assay	singlet oxygen	
Sample ID	Exhale coffee (repeat)	
Batch No.	Higham Miller Coffee Ltd	
Solvent	water	
Solvent control	18905	18954
Solvent control Mean	18929.5	
Standard Deviation	35	
%cv	**0.18**	
Y=1/2 Solvent control	9464.75	
X=EC50	0.06918887	
Sample Volume (uL)	**40**	
Total volume of well (uL)	**200**	
Dilution Factor	5	
EC50 Value	0.0138	mg/mL
ABEL-RAC™	7227	

CV of solvent control must be ≤ 3.0

ABEL-RAC™ using a LINEAR regression of 4 points

17/09/2020

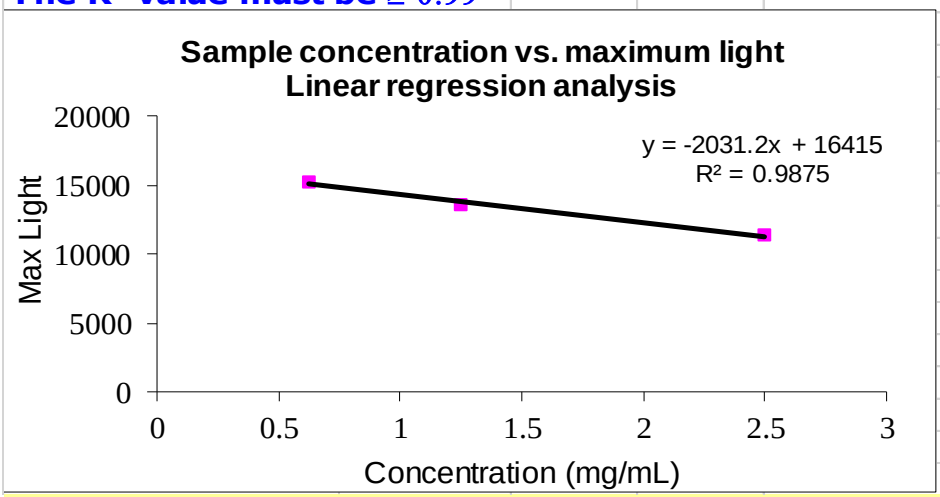
The EC_{50} is the concentration of a material that reduces the light by 50% compared to a solvent control. The EC_{50} value (mg/mL) of a sample is calculated from the equation of a linear regression analysis obtained from plotting the sample concentration (mg/mL) against peak light values taken from the light response curves. ABEL-RAC™ scores are calculated by multiplying the reciprocal of the EC50 value by 100

1. Copy and paste 'Maximum' values and enter sample concentrations (starting concs)

Sample	Maximum Light				
mg/mL	repl1	repl2	mean	% quenching	
Solvent control	17459	17636	17548		
5	8521	8511	8516	**51**	0.1
2.5	11383	11456	11420	**35**	0.5
1.25	13658	13599	13629	**22**	0.3
0.625	15259	15362	15311	**13**	0.5

2. Graph (plots 'Concentrations mg/mL' vs 'Maximum Light').The trendline, equation and R^2 value are automatically displayed.

The R^2 value must be ≥ 0.99



Intercept	16415
Slope	-2031.2

4. Evaluation template

This evaluation template is used to calculate the final ABEL-RAC™ score per mg of sample.
Input values highlighted in the table below for the sample size and total assay volume (µL)

Assay	singlet oxygen	
Sample ID	blueberries	
Batch No.		
Solvent	water	
Solvent control	17459	17636
Solvent control Mean	17547.5	
Standard Deviation	125	
%cv	**0.71**	
Y=1/2 Solvent control	8773.75	
X=EC50	3.76193876	
Sample Volume (uL)	**40**	
Total volume of well (uL)	**200**	
Dilution Factor	5	
EC50 Value	0.7524	mg/mL
ABEL-RAC™	133	

CV of solvent control must be ≤ 3.0

ABEL-RAC™ using a LINEAR regression of 4 points

17/09/2020

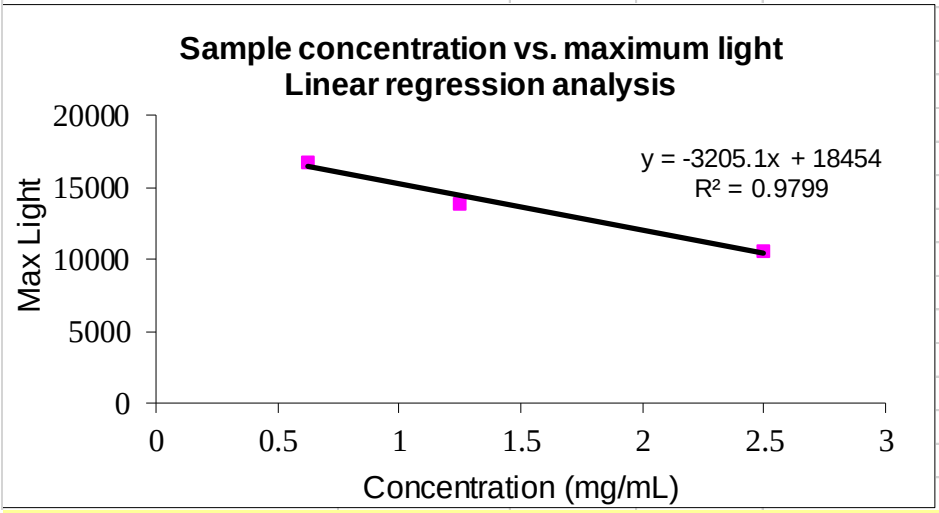
The EC_{50} is the concentration of a material that reduces the light by 50% compared to a solvent control. The EC_{50} value (mg/mL) of a sample is calculated from the equation of a linear regression analysis obtained from plotting the sample concentration (mg/mL) against peak light values taken from the light response curves. ABEL-RAC™ scores are calculated by multiplying the reciprocal of the EC50 value by 100

1. Copy and paste 'Maximum' values and enter sample concentrations (starting concs)

Sample	Maximum Light					
mg/mL	repl1	repl2	mean	% quenching		
Solvent control	20052	20248	20150			
5	6820	6789	6805	**66**	0.3	
2.5	10513	10700	10607	**47**	1.2	
1.25	13910	13992	13951	**31**	0.4	
0.625	16921	16642	16782	**17**	1.2	

2. Graph (plots 'Concentrations mg/mL' vs 'Maximum Light').The trendline, equation and R^2 value are automatically displayed.

The R^2 value must be ≥ 0.99



Intercept	18454
Slope	-3205.08571

4. Evaluation template

This evaluation template is used to calculate the final ABEL-RAC™ score per mg of sample.
Input values highlighted in the table below for the sample size and total assay volume (µL)

Assay	singlet oxygen	
Sample ID	curly kale	
Batch No.		
Solvent	water	
Solvent control	20052	20248
Solvent control Mean	20150	
Standard Deviation	139	
%cv	**0.69**	
Y=1/2 Solvent control	10075	
X=EC50	2.61420466	
Sample Volume (uL)	**40**	
Total volume of well (uL)	**200**	
Dilution Factor	5	
EC50 Value	0.5228	mg/mL
ABEL-RAC™	191	

CV of solvent control must be ≤ 3.0

ABEL-RAC™ using a LINEAR regression of 4 points

17/09/2020

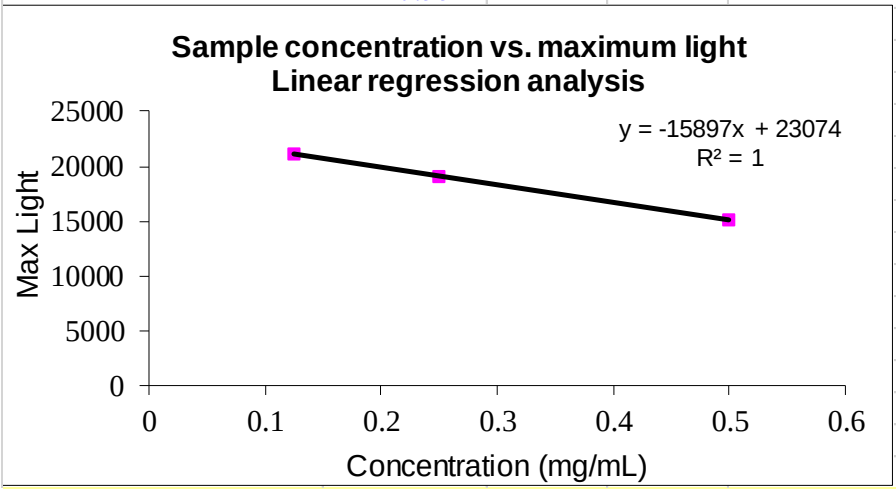
The EC_{50} is the concentration of a material that reduces the light by 50% compared to a solvent control. The EC_{50} value (mg/mL) of a sample is calculated from the equation of a linear regression analysis obtained from plotting the sample concentration (mg/mL) against peak light values taken from the light response curves. ABEL-RAC™ scores are calculated by multiplying the reciprocal of the EC50 value by 100

1. Copy and paste 'Maximum' values and enter sample concentrations (starting concs)

Sample	Maximum Light				
mg/mL	repl1	repl2	mean	% quenching	
Solvent control	23644	24434	24039		
1	11336	11316	11326	**53**	0.1
0.5	15269	14992	15131	**37**	1.3
0.25	19257	18908	19083	**21**	1.3
0.125	20787	21408	21098	**12**	2.1

2. Graph (plots 'Concentrations mg/mL' vs 'Maximum Light').The trendline, equation and R^2 value are automatically displayed.

The R^2 value must be ≥ 0.99



Intercept	23074
Slope	-15897.1429

4. Evaluation template

This evaluation template is used to calculate the final ABEL-RAC™ score per mg of sample.
Input values highlighted in the table below for the sample size and total assay volume (µL)

Assay	singlet oxygen	
Sample ID	freshly squeezed orange	
Batch No.		
Solvent	water	
Solvent control	23644	24434
Solvent control Mean	24039	
Standard Deviation	559	
%cv	**2.32**	
Y=1/2 Solvent control	12019.5	
X=EC50	0.69534508	
Sample Volume (uL)	**40**	
Total volume of well (uL)	**200**	
Dilution Factor	5	
EC50 Value	0.1391	mg/mL
ABEL-RAC™	719	

CV of solvent control must be ≤ 3.0

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