Dynamic Test Kits for R&D and Quality Control

Instruction manual

eBOC lab

BioQuChem

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DECLARATION OF CONFORMITY

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We:

certify that the product e-BQC lab has been built and has undergone final type testing according to the standards:

Electromagnetic Compatibility	EN/IEC 61326-1:2006 EN/IEC 55011:2011/A1:2011, EN/IEC 61000-4-2:2010, EN/IEC 61000-4 -3:2007/A2:2011

This instrument meets the requirements of the CE mark as contained in the EU directives 2006/95/EC (LVD), 2004/108/EC (EMC). It fulfills the following specifications:

EN 61326-1 Electrical equipment for measurement, control and laboratory use – EMC requirements

Oviedo, Junio de 2018

Bioquochem, S.L., Oviedo, Spain, will not accept any liability for damages caused directly or indirectly by connecting this instrument to devices which do not meet the relevant safety standards. The reader is designed as a laboratory research instrument for use with electrochemical sensors. BQC cannot, under any circumstance, be held responsible for the outcome or interpretation of data measured with eBQC

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1 Warnings and Precautions

Read carefully this instruction manual before starting the tests.

General Warnings

Keep the device out of reach of children.

Avoid exposing e-BQC disposable strips as well as the e-BQC device to excessive humidity, heat, cold, dust or dirt. Preferably, store the device inside its box, to avoid possible damage.

Do not touch the disposable strips without globes and avoid touching the area destined to the sample in the e-BQC disposable strips.

Keep the strips in their original container.

WARNING: Potential Biohazard and Safety Instructions

Always dispose of the used e-BQC strips as medical waste.

CAUTION: Use of the e-BQC device

The e-BQC device works ONLY with e-BQC disposable strips.

Do not use an e-BQC disposable strip that appears to be damaged or has been used.

2 e-BQC lab characteristics

e-BQC lab presents a new technology for antioxidant capacity measurement.

- Portability: Portable device for a quick and easy measurementanywhere.

- Ease of use: No specialized technical personnel is required.

- Accuracy: Two values (fast and slow antioxidants).

- Simplicity: No laboratory is needed. You only need one device for all your measurements.

- Quickness: The result is obtained in less than a minute and with a single drop of sample.

3 e-BQClab presentation

The e-BQC lab is a portable device, patent pending, designed to measure, in a direct way, the antioxidant capacity of plasma, with extensive application to other aqueous liquid samples. The result is obtained in less than a minute and with just one drop of sample. The antioxidant capacity is a parameter that measures the overall mechanisms that living organisms use to fight against oxidative stress (see Scheme 1).

Oxidative stress is caused by an imbalance between oxidant and antioxidant substances in favor of the first ones, which causes several pathologies.

Each antioxidant presents a different mechanism of action.

This mechanism depends on their oxidative potential, which is the energy required for the antioxidant to be oxidized. For this reason, the sooner the antioxidant is oxidized (lower oxidative potential), the more efficient it is fighting against oxidative stress, protecting other molecules with higher oxidative potentials from being oxidized.

E-BQC lab is based in electrochemistry, the science that studies the reactions of oxidation and reduction of molecules.
Electrochemistry is one of the most powerful analytical techniques developed. It is characterized by its high sensitivity and reproducibility. The use of the electrochemistry has been widely spread through the scientific community, growing exponentially in the last few years and being featured in more than nine thousand papers only in 2018.

By means of disposable e-BQC strips, e-BQC lab performs a complete oxidation of the sample, and so, it is able to show a precise and reliable measurement.

Scheme 1. Representation of the antioxidant barrier against oxidative stress, that prevents damage to lipids, proteins and DNA.

4 e-BQC lab description

E-BQC lab is a hand-held customized Potentiostat to be used with disposable strips: the e-BQC strips. The instrument contains a microprocessor which controls the potential applied to the sensor and measures the current (µC) that passes through the sample.

4.1 What does the e-BQC lab device contain?

- e-BQC device: dimensions 8,5x6x2,5 cm
- Power adapter
- USB cable (USB-mini USB)

NOT included: e-BQC strips (required to perform the test)

Figure 1 e-BQC lab box with its components

4.2 Front Panel

e-BQC lab device works directly connecting e- BQC disposable strips to the instrument, through the slot located on the Front Panel (Figure 2).

Figure 2. e-BQC lab Front Panel

4.3 Rear Panel

The rear panel has a USB connection for battery charging (Figure 3). USB cable is included.

Figure 3. e-BQC lab Rear Panel

4.4 Upper panel

e-BQC lab Upper Panel counts with an LCD Screen and a button (Figure 4).

Figure 4. e-BQC lab Upper Panel

Button operation

When the instrument is switched off, press to SWITCH ON.

When the instrument is switched on, press long to SWITCH OFF.

4.5 Power Supply

e-BQC lab incorporates a Li-ion Battery (1250 mAh), that can be charged using the USB cable-power adapter (included).

We recommend not to drain the device battery.

5 e-BQC lab measurement

The e-BQC lab portable device is based on a REDOX potential measure, that is expressed in charge units- micro-Coulombs (µC).

The e-BQC lab can distinguish between the differenttypes of antioxidants present in plasma, from the weakest to the strongest ones. E-BQC lab distinguish between Q1 and Q2, which gives an idea of the antioxidant capacity due to fast and slow antioxidants.

Antioxidants	Examples
Fast Acting Antioxidants	Uric acid, ascorbic acid (vitamin C), GSH, vitamin E, CoQ10, carotenoids...
Slow Acting Antioxidants	Polyphenols, Alpha-lipoic acid (ALA), resveratrol, astaxanthin...

Table 1 Examples of fast and slow acting antioxidants in human plasma samples based on their oxidation potential.

The different antioxidants are shown as two values of charge:

- Q1: Refers to the antioxidant capacity of the compounds with the highest rate of free radical scavenging. These are the fast acting antioxidants which are oxidized in the first place. These can be considered more powerful tan slow-acting antioxidants although they are present in less concentration.

- Q2: Refers to the antioxidant capacity of the compounds with a lower rate of free radical scavenging. These are the slow acting antioxidants.

With this two values, the e-BQC lab device calculates a QT, which is the sum of both.

Type of sample	Q1	Q2	QT
Blood	5-15	8-20	13-25
Plasma	5-15	15-25	20-45
Urine	90-140	100-140	190-280
Saliva	10-25	20-35	30-60

Table 2 Example values of Q1, Q2 and Qt in Human samples. These values are merely informative, and they can be affected by multiple factors.

6 How to use the e-BQC lab

6.1 What do you need to perform the test

- e-BQC lab device
- e-BQC strips (not included): plastic support strips covered with inert carbon material. Single use only.

- Sample: aqueous liquid samples. (*Ask BQC for non-aqueous samples.)

6.2 Before beginning the procedure

- Check the batterylevel.
- Prepare the e-BQC strips needed to perform the test.

6.3 Getting the results

The instrument displays the instructions on the screen to guide the user. The complete instructions are described below.

Step 1: Switch ON e-BQC lab. The welcome screen is displayed (Figure 5a), automatically the initial instructions will appear on screen (Figure 5b).

Step 2: If you want to start a new measurement, please insert a Disposable Strip into the slot located on the Front Panel, drop the sample onto the disposable strip, as indicated on Figure 6a and b, and press the button. Then, the display on Figure 5c is shown.

Press the button to start a new measurement or, on the contrary, after 10 seconds, the instrument will show the last measurement (Figure 5d).

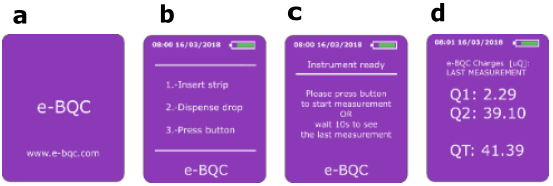

Figure 5. E-BQC screenshots showing welcome screen (a), initial instructions (b), optional access to the last measurement (c) and an example of last measurement display (d).

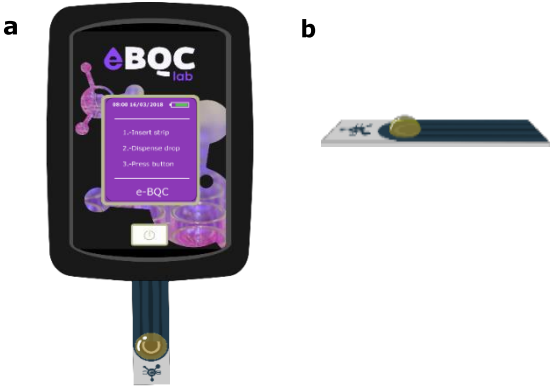

Figure6.a)e-BQClabinstrumentwithadropofsample
b) Side view of the strip, where the approximate recommended size of the sample drop is shown.

Step 3: If the button has been pressed, messages described on Figure 7 will be shown. The strip cannot be extracted until it is indicated on the screen (Figure 7).

Figure 7. Screenshot sequence displayed during measurement.

Step 4: Reading results. When the measurement is finished, the Antioxidant Capacity is displayed on the screen, expressed as Q1, Q2 and QT (µC)(Figure 8).

If unit conversion is needed, a calibration curve must be performed, as it is shown in Point 7- Calibration curve example.

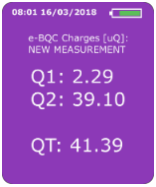

Figure 8. New Measurement results screenshot showing Q1, Q2 and QT (µC).

Step 5: If another measurement is going to be performed, extract the strip and press the button to restart. Then follow the instructions from step 2.

Step 6: Once the strip has been extracted, the instrument can be switched off by pressing long the button. After a few minutes, if the button is not pressed, e-BQC lab automatically will switch off (Figure 9).

Figure 9. Screenshot of final display.

7 Calibration curve examples

If unit conversion is needed to express the antioxidant capacity in equivalents of a model antioxidant, like ascorbic acid equivalents (CEAC), a calibration curve should be performed.

Here there are two proposals, a calibration curve for pure compound solutions; or the standard addition method for complex matrices.

7.1 Calibration Curve

To make this calibration curve example, ascorbic acid was used as a standard. Different concentrations of the standard were measured with the e-BQC device, the values were represented and the standard curve was created performing a linear regression, as shown in Figure 10.

For example, if a calibration in equivalents of ascorbic acid (CEAC) is required, the ascorbic acid solution must be prepared in the same conditions as the samples to be analyzed (buffer, pH, etc.)

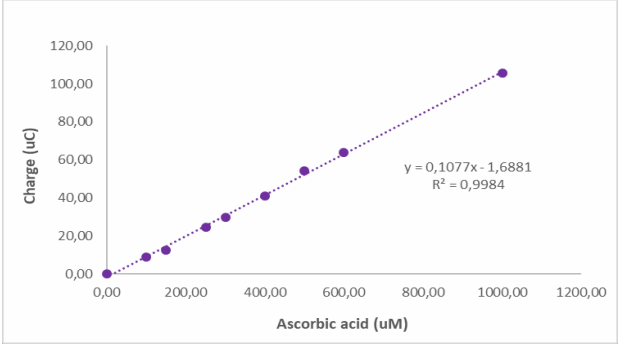

Figure 10. Example of a calibration curve that represents charge (uC) against concentration of ascorbic acid in phosphate buffer 0.1M (pH 5.8). Each point was measured in triplicate and the average was represented.

7.2 Standard Additions.

In order to obtain a calibration curve from complex matrix samples, it is recommended to follow the standard addition method, as it is shown in Figure 11. It shows a linear regression obtained from the representation of the final concentration of ascorbic acid, after adding a constant volume (1 µL) of increasing concentrations of ascorbic acid to 29 µL of plasma, against QT . The antioxidant capacity of the sample, expressed in equivalents of ascorbic acid (CEAC), is obtained by calculating the cutoff point of the calibration curve with the abscissa axis.

This method should be performed for each different sample to eliminate matrix effect.

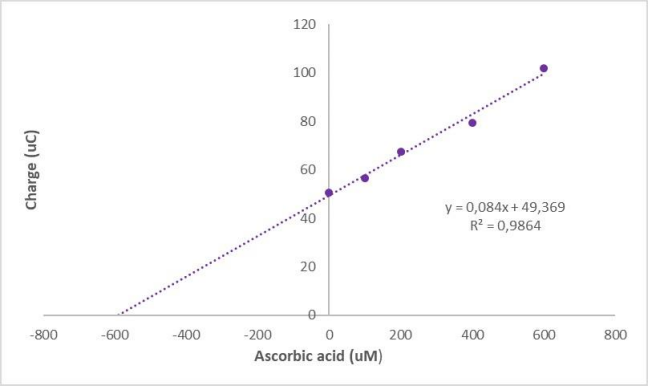

Figure 11. Standard Additions calibration example with ascorbic acid in a human plasma sample.

8 Correct handling of simples

As the device performs an electrochemical measurement, which is extremely sensitive, of the antioxidant capacity, minimal variations in conditions can affect the measurement (CV around 10%). Therefore, some recommendations should be taken into consideration:

- Temperature control: Variations in the temperature can affect the results of the measurements since these changes can damage the stability of the antioxidants present in the sample. Before performing the experiment, make sure that all the samples are at the same temperature. It is recommended to perform measurements with samples at room temperature.

- Air exposition: Avoid prolonged air contact/exposure of the sample, to prevent oxidation.

- Avoid using high concentrated saline buffers to treat your samples, as it may affect the results.

- Avoid pH variations between replicates of an experiment, as it may interfere with the results.

9 General recommendations

- Remember to write down the results shown on the screen. Each time the device is turned off, memory resets, so you can only see the last measurement while the device is on.

- Drop sizes between 30-50 µL are recommended. Volume will depend on the viscosity of the sample, since it may be necessary to put a larger volume to cover the strip completely, as shown in Figure 6b-c. In any case, avoid using less than 30 µL, as they could get incorrect readings. Keep this volume constant throughout your experiments, in order to compare the results of the measurements between tests.

- In order to optimize repeatability it would be advisable to use a buffer solution (i.e. phosphate buffer) to suspend or dilute your samples.

- Do not make a measurement without sample on the disposable strip, it will give an ERROR alert and the strip will be useless. If the button is pressed without a disposable strip inserted on the device, it will give an ERROR alert.

- Do not drain e-BQC lab battery.

10 Storage and Cleaning

- Handle the e-BQC lab device with care; do not drop.
- Do not expose the device or the supplies to high humidity, extreme heat, cold, dust or dirt.
- Do not scratch or damage the surface of the e-BQC disposable strips.
- Avoid fouling the device slots, as it can affect the measurements and even damage the device.
- Whenever possible, keep the instrument and accessoires inside the box.
- The case of the device can be cleaned with a lint-free cloth or a mild disinfectant solution.

CAUTION: When cleaning, do not allow liquids to spill through the button or slots.

11 Warranties and Limitation of Liability

Please ensure that you have carefully read and understood the conditions of this Warranty.

BIOQUOCHEM S.L. (Edificio CEEI, Parque Tecnológico de Asturias, 33428 Llanera, Asturias, Spain), warrants to the original purchaser only, that the e-BQC lab edition device will be free from defect in materials or workmanship for a period of two years from the date of original purchase.

If during this time the device does not work properly because of a defect in materials or workmanship, BIOQUOCHEM reserves the right of repairing or replacing it with another Remanufactured Product or replacing the unit for a new one. BIOQUOCHEM will pay for parts, labor and any shipping both ways to and from the Customer's site.

Customer claiming warranty should provide an original invoice as proof of purchase.

This warranty does not apply to the performance of e-BQC device that has been damaged by accident or has been altered, missused, tampered with or abused in any way.

This warranty only applies to the original purchaser of the device and/or its agents. The warranty of the repaired/replacement device will expire on the date of the original warranty expiration or ninety (90) days after shipment of a replacement system, whichever period is longer.

All services are subject to a valid defect being identified within the Product. If the damage or defect is not covered by this Warranty or subject to statutory rights, the Customer will be charged for the

cost of inspection or repair of the good, including all time and partsspent.

This warranty is in lieu of all other warranties, expressed orimplied,includinganyimpliedwarranty of merchantability or fitness for any purpose, other than stated herein

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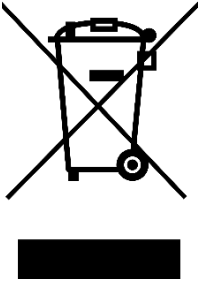
Note: Removing or loosening screws from the back ofthe analyzer voids all warranties. There are no user serviceable parts inside the case.



Processing of electric and electronic equipment at the end of their service

This symbol, affixed to the product or its packaging, indicates that the product must not be processed with household waste. It must be brought to an electric and electronic waste collection point for recycling and disposal. By ensuring the appropriate disposal of this product you also help in preventing potentially negative consequences for the environment and human health. The recycling of materials helps preserve our natural resources. For further information regarding the recycling of this product, please contact your municipality or local waste disposal center.

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