BRS

BQC REDOX SYSTEM

New analytical device to assess the redox state of **biomedical samples**

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BRS DEVICE

BRS (BQC Redox System) is the first **multiparametric portable** device **based on electrochemistry** designed to assess the redox state of biomedical samples. With one single device, it is possible to determine different markers of the redox state in a simple, fast and precise way.

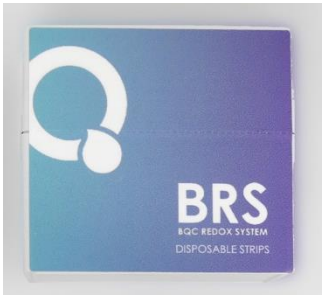
The device is now available and ready for the measurement of **Total Antioxidant Capacity** (TAC) and **H_2O_2 Scavenging Activity** in a wide variety of biological samples.

The device has been designed for a continuous upgrading and improvement through the incorporation of new redox measurements.

BRS performance has been validated in **cell cultures, human blood, plasma, serum, erythrocytes, saliva and urine samples.**

The technology behind the BRS for **TAC** determination is protected by a granted utility model. **H_2O_2 Scavenging Activity** measurement is currently patent pending.

BRS TEST STRIPS



BRS device works exclusively **with BRS disposable strips.** Two different types of test strips for measuring TAC or H_2O_2 Scavenging Activity are available.

TAC Strips. Plastic support test strips covered with a carbon material, specially designed for TAC measurement.

H_2O_2 Strips. Ceramic support test strips covered with a modified carbon material that exhibits electrocatalytic properties towards H_2O_2 oxidation.

BRS is a new electrochemical, multiparametric and portable device designed to assess the global redox state of different type of biological samples.

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WHY TO USE BRS?

- ✓ **Simple, direct and fast analysis**
- ✓ **Easy operation**
- ✓ **Low volume sample**
- ✓ **No need of lab equipment**
- ✓ **No modification of sample native conditions**
- ✓ **No complex sample pretreatment**
- ✓ **Suitable for color and turbid samples**

HOW TO USE BRS DEVICE

The **use** of the BRS device is very **simple**. Insert the test strip into the device, place a drop of sample onto the test strip, and start the measurement.

The device is controlled by a specially designed App to use on your mobile device. **BRS App** functions include running the measurements and storing and sharing the data. In approximately 1 minute results are displayed on your mobile device.

Some Technical data

Weight

±250 g

Power supply battery

>6 hours continuous measurements

Communication

Bluetooth

Temperature range

0 °C to +40 °C

Measuring range

0-4000 µC

WHAT IS OXIDATIVE STRESS?

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) and the ability of the antioxidants present in an organism to fight them. Oxidative stress is responsible for ageing and has been related to different pathologies like obesity, diabetes, cancer, and neurodegenerative disease.

The balance between ROS/RNS and antioxidants is critical in maintaining healthy biological systems.

Exposure to pathogens, bad life style, smoking, pollution, UV light or malnutrition are some of the factors that can promote the generation of ROS/RNS. Fortunately, tissues and cells are protected against ROS/RNS damage by an antioxidant defense system. This defense system includes endogenous antioxidants (enzymatic and non-enzymatic) and exogenous antioxidants (dietary antioxidants). Despite this efficient antioxidant system, under some conditions, the balance between the production of ROS/RNS and the antioxidant system is broken, causing an oxidative stress state.

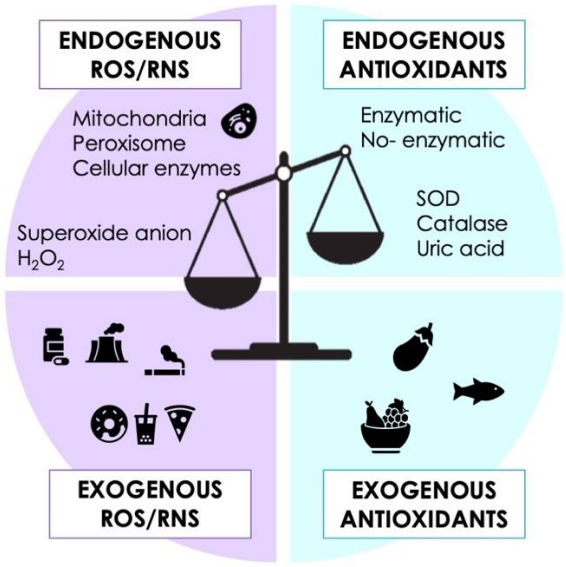


Figure 1.*Schematic representation of Oxidative Stress*

Due to the transient nature of ROS/RNS, their measurement in biofluid samples is very difficult. By this reason the antioxidant capacity of body fluid is usually measured as an indirect marker of the oxidative stress state.

WHY IS IMPORTANT TO STUDY OXIDATIVE STRESS?

Oxidative stress induces extensive modifications or damage to macromolecules (DNA, lipids and proteins) that can induce cell death and tissue injury. Oxidative stress is also implicated in the onset, progression and pathogenesis of numerous diseases and disorders such as infertility, obesity, diabetes, cancer, Alzheimer, etc.

Determining the degree of **oxidative stress** is therefore of **great interest** to study:

- Disease prevention, control and treatment.
- Aging process.
- Effect of lifestyles (diet, sport) on health.

WHY ELECTROCHEMISTRY TO STUDY OXIDATIVE STRESS?

Redox reactions are involved in the origin and consequences of oxidative stress. Electrochemistry is recognized as the best tool to study this type of reactions. Another important aspect in favour of the electrochemical methods to study oxidative stress, is the electroactivity of most of the oxidants and antioxidants present in biological samples.

Electrochemistry is therefore a powerful tool to study oxidative stress in biomedical samples, with features that include rapid response, high sensitivity, inherent miniaturization, low cost and low-power requirements. Moreover, electrochemical techniques are non-destructive and can be used in turbid or colored samples.

BRS is the first electrochemical based device designed to study oxidative stress markers in biomedical samples.

BRS allows a simple and fast measurement of biomedical samples redox state by measuring Total Antioxidant Capacity and Hydrogen Peroxide (H_2O_2) Scavenging Activity.

TOTAL ANTIOXIDANT CAPACITY (TAC)

WHAT IS TAC?

Total antioxidant capacity (TAC) is a global measurement of the non-enzymatic antioxidant defense system that integrates the individual effect of all antioxidants in a given matrix, and their additive, synergistic or antagonistic interactions. **TAC is considered an important parameter to establish the redox state of biological samples** and can be used as an **oxidative stress biomarker**.

METHODS FOR TAC DETERMINATION

Due to the lack of standard quantification methods for TAC determination a wide variety of assays have been used to asses the antioxidant capacity of biomedical samples. Most popular TAC assays are based on the spectrophotometric monitoring of reactions between antioxidants and specific oxidants or radicals generated *in situ*.

TAC assays may be broadly classified as the electron transfer (ET)- and hydrogen atom transfer (HAT)-based assays. Briefly, HAT-based assays measure the capability of an antioxidant to quench free radicals by H-atom donation while ET-based assays measure the capacity of an antioxidant in the reduction of an oxidant. The most popular HAT-based TAC assay is the fluorometric oxygen radical absorbance capacity (ORAC). Ferric reducing antioxidant capacity (FRAP), cupric reducing antioxidant capacity (CUPRAC), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radical scavenging assay (ABTS) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate radical scavenging assay (DPPH) are the most frequently used ET-based TAC assays. ABTS and DPPH can also be considered as mixed-mode HAT/ET.

The results obtained from the different TAC assays are hardly comparable because of the different mechanisms, pHs and solvents required for each assay.

Independently of the mechanism, **spectrophotometric TAC assays are usually quite complex, time consuming due to the requirement of sample pretreatmen (e.g. deproteinization) and rely on expensive instrumentation.**

Focussing on biological samples, ORAC is the only radical based TAC assay that use a biologically relevant radical. However, ORAC assay is very complex and requires a very careful control of temperature, reagent concentrations, etc. Although more simple than ORAC, DPPH and ABTS assays do not use physiological relevant radicals. Regarding the non radical based TAC assays, the redox reaction producing coloured species in CUPRAC is carried out at nearly physiological pH as opposed to the unrealistic acidic conditions of FRAP. The main limitations of CUPRAC are the need of organic solvents and sample pretreatment.

Table 1. *Comparison of methods for TAC determination.*

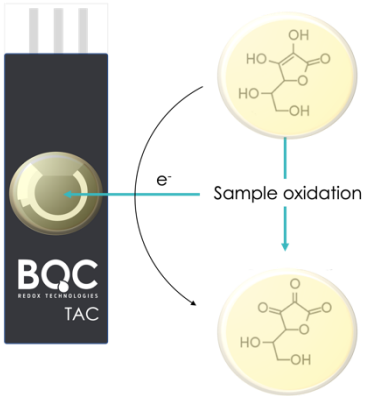
TAC Spectrophotometric Method		BRS
Method	**Main Limitations**	**Advantages**
ORAC	Complexity	Simple
DPPH	Non-physiological radical	No radical/oxidant
ABTS	Non-physiological radical	No radical/oxidant
FRAP	Non physiological pH	Direct sample analysis
CUPRAC	Organic solvents	No organic solvents
All methods	Interferences from colored/ turbid samples	Suitable for colored/turbid samples
	Time consuming	Fast (~ 1 min)
	Lab equipment	No lab equipment

BRS overcomes main limitations of spectrophotometric TAC methods and provides additional information about the electron transfer ability of antioxidants.

BRS DEVICE FOR TAC ANALYSIS

BRS electrochemical device provides a rapid, simple and sensitive alternative to spectrophotometric assays for TAC determination.

BRS uses a voltammetric technique to measure TAC. The sample is electrochemically oxidized by applying a potential scan and TAC value is calculated from the voltammetric charge.



Similar to ET spectrophotometric TAC assays the BRS measurement is based on an electron transfer process from the antioxidants to the electrode.

Different from ET spectrophotometric TAC assays that requiere sample modification, **BRS analysis can be considered as a direct evaluation of the antioxidant activity of the sample in its native environment**.

The technology behind the BRS for TAC determination is protected by a granted utility model.

BRS analysis does not require toxic reagents and even **colored or turbid samples** can be analyzed.

BRS TAC results are expressed in terms of **electric charge (µC)** as **Q_1, Q_2 and Q_T.** BRS device, thanks to electrochemistry, also **provides information about how easy or difficult is to oxidize the antioxidants present in a sample.**

Q_1. Antioxidant capacity related to low redox potential antioxidants. Easy electron transfer/High antioxidant activity.

Q_2. Antioxidant capacity related to high redox potetial antioxidants. More difficult electron transfer/Low antioxidant activity.

Q_T. Total antioxidant capacity (**Q_1+ Q_2**).

TAC measurements obtained with **BRS** device in Q_T (µC) can be easily converted into Trolox Equivalents (**TEAC**, Trolox Equivalents Antioxidant Capacity), Vitamin C Equivalents (**CEAC**, Vitamin C Equivalents Antioxidant Capacity) or Gallic Acid Equivalents (**GAE**) by simply performing calibration curves with the device using Trolox, Ascorbic Acid or Gallic Acid as standard.

BRS ANALYTICAL CHARACTERISTICS FOR TAC ANALYSIS

LINEAR RANGE

BRS exhibits a **wide linear range** for the most used standards of TAC. All the standards were prepared in 0.1 M PB pH 7. Thanks to this wide linear range no sample dilution is required for the analysis of most of the samples. If sample dilution is needed, it is recommended to use 0.1 M PB pH 7.

Table 2. *BRS linear range for TAC standards.*

TAC Standard	BRS Linear range (µM)
Ascorbic Acid	50-10000
Trolox*	50-10000
Gallic Acid	25-10000

***Linear range for TROLOX spectrophotometric detection (BQC Assay Kits):**

ORAC, 10-175 µM

ABTS, 50-600 µM

DPPH, 100-500 µM

CUPRAC, 250-2000 µM

PRECISION

Intra-assay precision was evaluated by replicate analysis (n=10) of a human serum sample using a new strip for each measurement. Inter-assay precision was calculated by measuring a standard solution (100 µM Ascorbic Acid) on ten different days using a new strip for each measurement.

BRS analysis shows good precision with intra and inter-assay coefficients of variation (CV) **< 10 %.**

Table 3. *BRS precision.*

Precision (n=10)	Q_T /Mean Value ± SD	CV (%)
Intra-assay (Human serum)	51± 4	8
Inter-assay (Ascorbic Acid 100 µM)	27 ± 2	7

BRS TAC SAMPLE ANALYSIS

Table 4 shows the TAC values obtained for several biological samples (healthy donors). Samples were measured directly without any further dilution. Red bloos cells were lysed by adding four times their volume of ice cold ultra-pure water followed by a centrifugation step at 10000 x g for 15 min at 4° C.

Table 4. *BRS TAC values for biomedical samples.*

Sample	Q_T (µC)*
Saliva	30-150
Urine	250-1000
Plasma	40-50
Serum	40-60
Whole Blood	30-65
Lysed Red Blood Cells	30-40

***Do not use as reference values**

H_2O_2 SCAVENGING ACTIVITY

WHY IS IMPORTANT TO MEASURE H_2O_2 SCAVENGING ACTIVITY?

Hydrogen peroxide (H_2O_2) is formed in cells by controlled pathways and trigger a broad spectrum of cellular response. Locally intense amounts of H_2O_2, produced for example by inflammatory cells, can also cause oxidation of proteins, lipids and DNA.

Despite its poor reactivity, H_2O_2 in the presence of transition metal ions forms the extremely reactive hydroxyl radical by Fenton chemistry. H_2O_2 can also selectively oxidize some methionine and cysteine residues in certain proteins and is a substrate for myeloperoxidase, generating further reactive species such as HOCl. Scavenging H_2O_2 from biological samples is therefore necessary for protection against oxidative damage. Main scavengers of H_2O_2 in biological samples include enzymes such as catalases, glutathione peroxidases and peroxiredoxins.

H_2O_2 Scavenging Activity can be used to assess the antioxidant capacity (enzymatic and non-enzymatic) of a biological sample. The greater the scavenging activity of H_2O_2, the greater the antioxidant capacity.

H_2O_2 Scavenging Activity is an important parameter to monitor the ability of a sample to eliminate ROS and prevent damage.

METHODS FOR H_2O_2 SCAVENGING ACTIVITY DETERMINATION

Most popular H_2O_2 Scavenging assays are based on direct UV absorption measurement (230 nm) of hydrogen peroxyde with/without incubation with scavenger. Main limitation of these methods is that they are highly open to interferences from many UV-absorbing substances. Another common approach is to use fluorometric probes. However, most of these methods require the use of peroxidase enzymes that can also react with H_2O_2 scavengers. Chemiluminescent methods have also been described for H_2O_2 determination. Although very sensitive, these methods are expensive and require sophisticated laboratory tools.

BRS overcomes main limitations of optical H_2O_2 Scavenging assays.

BRS DEVICE FOR H_2O_2 SCAVENGING ACTIVITY ANALYSIS

BRS electrochemical device provides a rapid, simple and more reliable alternative to optical assays for H_2O_2 Scavenging Activity determination.

BRS H_2O_2 Scavenging Activity measurement is based on the electrochemical oxidation of H_2O_2 in the absence and presence of scavengers. BRS uses a voltammetric technique to measure the anodic current related to H_2O_2 oxidation using a specially designed test strips. H_2O_2 test strips are manufactured using a modified carbon material that exhibits electrocatalytic properties towards H_2O_2 oxidation.

Table 5. *Comparison of methods for H_2O_2 Scavenging Activity determination.*

Optical Methods H_2O_2 Scavenging Activity		BRS
Method	**Main Limitations**	**Advantages**
UV	Interferences	Selective
Fluorimetric	Interferences Require the use of enzymes	No enzymes
Chemiluminescent	Complex Expensive instrumentation	Simple Low cost instrumentation

BRS H_2O_2 Scavenging Activity is expressed as % using the following equation:

$$\%\ H_2O_2\ \text{Scavenging} = \frac{i_O - i_S}{i_O} \times 100$$

Where i_O is the current recorded for a known concentration of H_2O_2 and i_S is the current measured for the same H_2O_2 concentration in the presence of sample.

BRS H_2O_2 Scavenging Activity determination is a very simple two step procedure.

The electrochemical signal is recorded in less than a minute.

BRS ANALYTICAL CHARACTERISTICS FOR H_2O_2 SCAVENGING ACTIVITY DETERMINATION

PRECISION

Precision was calculated as intra-assay precision coefficient of variation (CV < 10%) by replicate analysis (n=10) of a 0.5 mM H_2O_2 solution, using a new strip for each measurement.

BRS H_2O_2 SCAVENGING ACTIVITY SAMPLE ANALYSIS

Table 6 shows the % of **H_2O_2 Scavenging Activity** calculated for several biological samples (healthy donors). Results are not directly comparable due to the different dilution of the samples. Samples were diluted in 50 mM PB pH 7. Red bloos cells were lysed by adding four times their volume of ice cold ultra-pure water followed by a centrifugation step at 10000 x g for 15 min at 4° C. Incubation time: 5 min at RT.

Table 6. *BRS H_2O_2 Scavenging Activity for biomedical samples.*

Sample (dilution)	H_2O_2 Scavenging (%)*
Saliva (1/10)	20-45
Urine (1/25)	10-35
Plasma (1/100)	25-45
Whole Blood (1/50)	25-65
Lysed Red Blood Cells (1/1000)	35-55

***Do not use as reference values**

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