

Analysis report ORAC Europe BV

*initials
investigator*

Customer name:

Fizziqs AG
att. Mr. W.J.A. Peters
Bahnhofstraße 21
6304 Zug
Switzerland

Amount of samples
delivered:

1 sample

EW

Date of sample arrival:

Tuesday, July 13, 2010

EW

Sample condition at arrival:

OK

Damaged

Other

(see remarks)

Sample storage conditions:

Upon arrival, sample was stored in the
dark at 4°C.

EW

Remarks:

Fizziqs AG sample was presented as a
dry powder, packaged in a sealed
plastic container.

EW

Customer's
description of samples:

Fizziqs AG

Fizziqs Complete - Stickpulver

07.07.2010

ORAC Europe
sample preparation:

Upon receipt, *Fizziqs AG* sample was coded as follows:

Fizziqs Complete - Stickpulver: 20100713-1

To further increase the homogeneity of the sample, sample was carefully ground using a porcelain mortar and pestle. The obtained fine, homogeneous powder was transferred to a screwcap-closed, brown glass bottle and stored in the dark at 4°C until further use.

Sample preparation:

To prepare a stock-solution, 1.0 gram of sample 20100713-1 was dissolved in 10.0 mL of freshly prepared phosphate buffer (pH = 7.4).

To enhance sample solubility, dimethyl sulfoxide (DMSO, max. 1%) was added to the weighed sample before the addition of phosphate buffer. This concentration of DMSO does not affect the measurement and outcome of the assay.

Sample was placed in a sonication bath for 10 min.

The sample dissolved completely.

After further diluting the stock-solution in phosphate buffer, each diluted sample solution was placed in a sonication bath for 10 min.

The stock-solution was diluted to an appropriate concentration for the hydrophilic ORAC assay shortly before the actual measurement.

Date of sample preparation:

Tuesday, July 20, 2010

EW

Required assay:

Hydrophilic ORAC assay

EW

Date(s) of testing:

Tuesday, July 20, 2010

EW

Responsible investigator:

Dr. E. van den Worm



EW

Report finalization date:

Tuesday, July 20, 2010

EW

Remarks:

- no remarks -

EW

Short description of performed assay:

Sample provided by *Fizziqs AG* was tested for its *Oxygen Radical Absorbance Capacity* (ORAC), using the commonly accepted and well-validated hydrophilic ORAC assay with fluorescein as fluorescent probe and with AAPH (2,2'-azobis (2-methylpropionamidine) dihydrochloride) as a physiological relevant peroxy radical generator. Kinetic fluorescence profiles were detected using an automated fluorescence reader (*Thermo Fluoroskan Ascent*). Fluorescence was monitored every minute for 1 hr. at 37°C, using an excitation wavelength of 485 nm and an emission wavelength of 538 nm. (Assay performed in accordance to: *Prior et al., J. Agric. Food. Chem.* 53(10): 4290-4302; 2005.)

In the ORAC assay, Trolox (a water-soluble derivative of vitamin E) is used as an internal standard. Therefore, the results of the ORAC assay are expressed as μmol Trolox equivalents (TE) **per gram of test-sample**. This is a standard way of expressing ORAC values.

Sample was dissolved and diluted in freshly prepared 75mM sodium phosphate buffer (pH = 7.4) shortly before the experiment. All reagents were freshly prepared prior to the experiment. All solutions were kept in the dark at 37°C except the AAPH solution which was kept on ice (in the dark) until use.

From the obtained experimental data, ORAC values were calculated using the 'area under the curve' (AUC). The net AUC was obtained by subtracting the AUC of the blank from that of the sample. The relative ORAC value (expressed as Trolox equivalents) was calculated by extrapolation from the Trolox calibration curve (AUC_{Trolox} vs. [Trolox]). ORAC values are expressed as mean values \pm Standard Deviation (S.D.).

Remarks:

After further dissolving the sample in phosphate buffer, sample was placed in an ultrasonication bath for 10 min. Before all dilution steps and final addition to test-plate, all sample dilutions were carefully vortexed.

TEST RESULTS:

ORAC Europe sample code	Customer ID	ORAC value (mean value ± standard deviation) (µmol TE / gram)*
20100713-1	Fizziqs Complete - Stickpulver	872.6 ± 49.4

** As stated, ORAC value is expressed as µmol TE **per gram** of test sample.
If required, customer can extrapolate these ORAC values
to µmol TE per serving or dosage unit.*

Responsible investigator:

Dr. E. van den Worm
(CEO, ORAC Europe BV)

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