



Greiner Bio-One Saliva Quantification Kit

Instructions for Use



1. Manufacturer

Greiner Bio-One GmbH, Bad Haller Straße 32, 4550 Kremsmünster, Austria

2. Intended Use

The Greiner Bio-One Saliva Quantification Kit is an *in vitro* diagnostic product exclusively intended for determining the saliva content in saliva samples, which are collected using the Greiner Bio-One Saliva Collection System. It is necessary to know the saliva content, in order to be able to determine the original analyte concentration.

Saliva collection using the Greiner Bio-One Saliva Collection System causes individual dilution of the collected saliva sample by rinsing out the mouth cavity with the saliva extraction solution, thus changing the original analyte concentration.

The Greiner Bio-One Saliva Quantification Kit does not permit the direct determination of analytes from saliva. Examinations from saliva, which give information on the composition of saliva, are only possible when used in combination with specific *in vitro* diagnostic products.

3. Product Description and Composition

Greiner Bio-One Saliva Calibrator 1	1 bottle; 4ml; 11U/mL*; ready for use, contains diluted saliva extraction solution, 1.9 mg/ml ammonium sulphate and 1mg/ml sodium azide
Greiner Bio-One Saliva Calibrator 2	1 bottle; 4ml; 27U/mL*; ready for use, contains diluted saliva extraction solution, 1.9 mg/ml ammonium sulphate and 1mg/ml sodium azide
Greiner Bio-One Saliva Calibrator 3	1 bottle; 4ml; 46U/mL*; ready for use, contains diluted saliva extraction solution, 1.9 mg/ml ammonium sulphate and 1mg/ml sodium azide
Greiner Bio-One Saliva Calibrator 4	1 bottle; 4ml; 66U/mL*; ready for use, contains diluted saliva extraction solution, 1.9 mg/ml ammonium sulphate and 1mg/ml sodium azide
Greiner Bio-One Saliva Calibrator 5	1 bottle; 4ml; 85U/mL*; ready for use, contains diluted saliva extraction solution, 1.9 mg/ml ammonium sulphate and 1mg/ml sodium azide
Greiner Bio-One Saliva Control 1	1 bottle; 4ml; 30U/mL*; ready for use, contains human saliva, saliva extraction solution, 1.9mg/ml ammonium sulphate and 1mg/ml sodium azide
Greiner Bio-One Saliva Control 2	1 bottle; 4ml; 70U/mL*; ready for use, contains human saliva, saliva extraction solution, 1.9mg/ml ammonium sulphate and 1mg/ml sodium azide

* 1U/mL corresponds to 1 volume percent of saliva

4. Precautions and Warnings

All kit components contain 1mg/ml toxic sodium azide. Contact to skin and eyes as well as swallowing should be avoided, and gloves should be worn. In case of skin or eye contact, the affected area should be rinsed thoroughly for 15 minutes with water. A doctor should be informed, if swallowed, or if the eye is affected. In combination with heavy metals, in particular lead or copper (waste pipes), sodium azide can form explosive metal azides. During disposal, care should be taken to avoid accumulations of azides forming, by rinsing out with plenty of water.

The saliva controls 1 and 2 contain human saliva which has been examined for the hepatitis B virus (HBV), hepatitis C (HCV) and human immunodeficiency virus type 1 (HIV-1), by means of nucleic acid detection, with the result "not detectable".

Due to a lack of reliable detection methods for excluding pathogens, the Saliva Controls must be viewed as potentially infectious material and may only be applied by trained personnel. Refer always to the instructions for use of the assay manufacturer for detecting the specific target of interest in the saliva sample.

5. Storage and Shelf-life

Storage: Protected from light at 4°C to 25°C; protect kit from frost, do not freeze.

Shelf-life: See label on packaging. After calibrators/controls have been opened, keep stored with the lid on at 4°C and use within 3 months.

6. Methodology

Samples collected with the Greiner Bio-One Saliva Collection System contain the food dye tartrazine as internal standard.

Using the saliva calibrator, the internal standard is determined photometrically and the saliva content of the saliva sample is back calculated.

7. Specimen Material

Only saliva samples that have been exclusively collected using the Greiner Bio-One Saliva Collection System can be analysed using the Greiner Bio-One Saliva Collection Kit.

8. Additional Materials and Laboratory Instruments Required

- Clinical analyser, cuvette photometer or plate-reading photometer with a measurement wave length of 450nm and 520nm (cuvettes, microtiter plates or similar, depending on requirements of photometers).
- If applicable variable micropipettes (100 – 1000µl) and disposable tips.

9. Preparation of Reagents and Examination Material

- Saliva samples must be at room temperature before beginning with the analysis.
- Before determining the saliva content, the Saliva Transfer Tubes containing the saliva samples must be centrifuged for 10 min. at 2200 g. The samples are not to be shaken after centrifugation. If shaking occurs, the centrifugation procedure must be repeated

10. Saliva Quantification Procedure

10.1 Calibration

Measure the saliva calibrators 1 – 5 at 450nm and 520nm and then determine the extinction difference by subtraction.

$$\Delta \text{Extinction}_{\text{calibrator}} = \text{Extinction}_{450\text{nm}}_{\text{calibrator}} - \text{Extinction}_{520\text{nm}}_{\text{calibrator}}$$

For clinical analysers, the system water functions as cuvette blank value.

For cuvette and plate photometers, it is recommended that deionised water be used as cuvette or plate blank value. Sufficient volume should be used for the measuring, depending on manufacturers' specifications or type of instrument. For cuvette photometers, 1cm cuvettes should be used. If a 96 well microtiter plate is used, it is recommended that 250µl is used for the measurement.

10.2 Measuring the samples

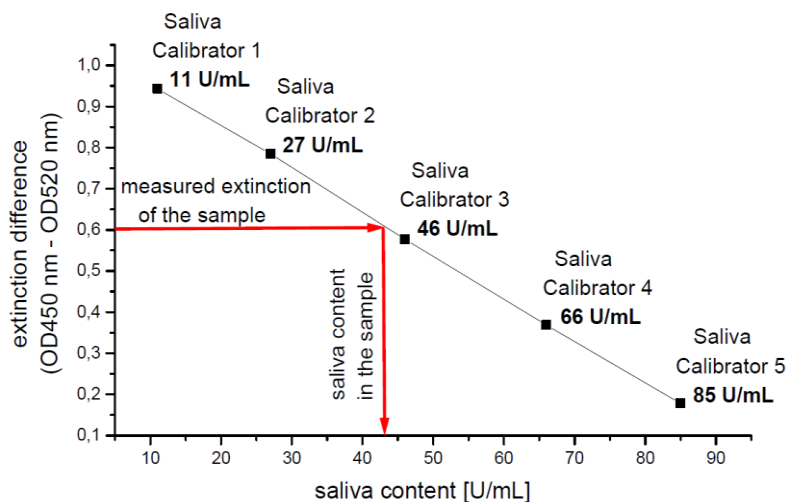
Subsequently the centrifuged saliva samples, which have been taken with the Greiner Bio-One Saliva Collection System, are measured using the same procedure as for the calibrators.

NOTE: Under www.gbo.com/preanalytics_protocols for automated determination of the saliva content for various analysers can be downloaded.

11. Calculation of the Saliva Content

By plotting the measured extinction differences against the calibrator units, a calibration line is given (fig. 1). Using the calibration line, the saliva content (U/ml) in the sample is calculated using the linear regression formula. The following is applicable: 1U/ml corresponds to 1ml volume percent of saliva.

Fig. 1: Example chart calibration and determination of a saliva specimen. This is an illustration and is not to be used for determination of patient samples.



12. Internal Quality Control

To ensure accurate determination, free of interference, the saliva controls numbered 1 and 2 (included in kit) must be determined prior to measuring one or more saliva samples. The resulting concentration must be within the given nominal value range (tab. 1). If the values are not within the nominal values range, the determination is invalid.

Tab.1: Nominal values of supplied saliva controls.

Control	Nominal values range (U/mL)
Saliva control 1	27 - 33
Saliva control 2	68 - 72

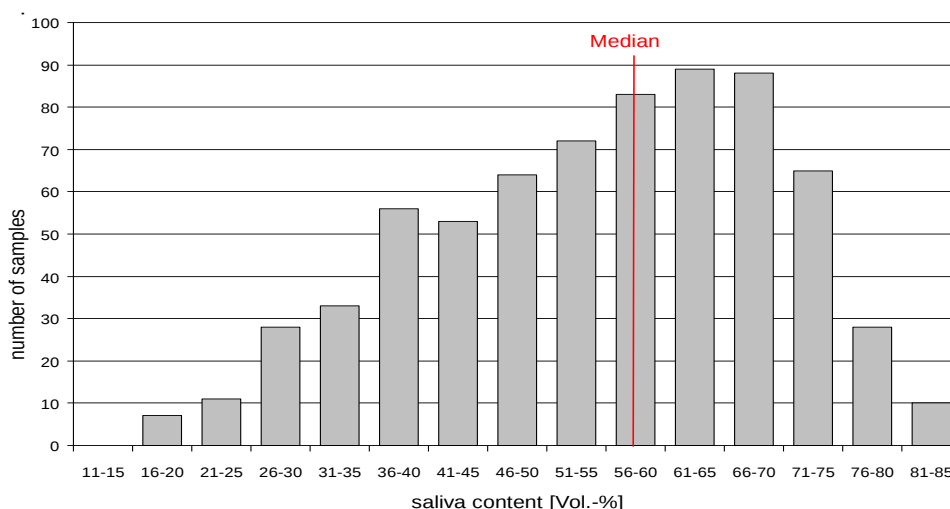
13. Performance Characteristics and Limitations

13.1 Analytical performance characteristics

13.1.1. Measuring range

The quantification range is between 11 and 85ml. Results outside of the quantification area should be dismissed as invalid.

Fig. 2 Distribution of saliva content amongst young persons and adults (age 15 – 92 years; n=687; ♀ : ♂ = 40% : 60%)



13.1.2 Accuracy

The deviation to each target value was determined by single determination of saliva samples with different saliva contents (tab.2) on a clinical analyser (Olympus AU400).

13.1.3 Accuracy of the mean

The deviation to each target value was determined by multiple determination of saliva samples with different saliva contents (tab.3) on a clinical analyser (Olympus AU400).

Tab.2: Accuracy.

Target value (U/mL)	Deviation to target value (%)
35	± 9
45	± 4
55	± 4
65	± 2
75	± 1

Tab.3: Accuracy of the mean.

Target value (U/ml)	Deviation to target value (%)
35	± 9
45	± 4
55	± 3
65	± 2
75	± 1

13.2 Analytical specificity, matrix interferences and limitations of the method

Impurities due to remains of food or blood in the extracted saliva sample can cause false analysis results. In this respect, caution should be taken even with food colouring in sweets, drinks or medication. The type of error that occurs depends on the type and amount of impurity. If the saliva sample is collected correctly using the Greiner Bio-One Saliva Collection System, this form of matrix interference does not normally occur. Samples with a high level of impurity after centrifugation or strong discolouration are to be discarded in view of incorrect results.

14. Subsequent Determination of Analytes and Assay Compatibility

Saliva samples collected with the Greiner Bio-One Collection System are in a matrix, which is not comparable with serum/plasma or urine. Test show, that many established methods of determination for detecting analytes from serum/plasma or urine must be adapted to get valid analysis results.

The system does not permit determination of the following analytes: BUN, urea, and sodium.

Previous experience has shown, that it is not possible to validly determine potassium and chloride using ion selective electrodes (ISE) and that furthermore, inhibitions can occur when detecting viral nucleic acids.

15. Label Information

	Manufacturer		Temperature limit
	Use-by date		Batch code
	Catalogue number		

16. Literature

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- Haeckel R. Walker RF. Colic D. Reference ranges for mixed saliva collected from the literature. J Chem Clin Biochem. 1989 Apr;27(4):249-52.
- Thomas L. Labor und Diagnose. Frankfurt/Main. TH-Books, 6. Aufl. 2005.
- Raggam RB et al. Evaluation of a novel standardized system for collection and quantification of oral fluid. Clin Chem Lab Med. 2008;46(2):287–291

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