Product information

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Alpha-Amylase Saliva ELISA

RUO **For Research Use Only – Not for Use in Diagnostic Procedures**

REF **IB79320**

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Version 11/20
DMC
Updated 210816

CONTENT

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1. INDICATION

The enzyme immunoassay (ELISA) provides determination of alpha amylase in human saliva.
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2. PRINCIPLE OF THE TEST

The test kit contains microplate strips each with 8 break-off reagent wells coated with anti-rabbit antibodies. In the first reaction step, diluted samples are pipetted into the reagent wells together with peroxidase-labelled alpha-amylase and a specific rabbit anti-alpha amylase antibody. Alpha amylase from the sample and the labelled alpha amylase in the conjugate compete for the free binding sites of the specific antibody. In the third incubation step, the bound peroxidase catalyses a colour reaction with the peroxidase substrate tetramethyl benzidine (TMB). The intensity of the colour formed is inversely proportional to the concentration of alpha amylase in the sample. The results for the samples are determined using the standard curve.

3. CONTENTS OF THE TEST KIT

Component	Colour	Format	Symbol
1. Antibody-coated microplate wells 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	SORB | MT
2. Calibrator 1, 0 U/ml, ready for use	light red to dark red	1 x 1.0 ml	CAL 1
3. Calibrator 2, 10 U/ml, ready for use		1 x 1.0 ml	CAL 2
4. Calibrator 3, 30 U/ml, ready for use		1 x 1.0 ml	CAL 3
5. Calibrator 4, 80 U/ml, ready for use		1 x 1.0 ml	CAL 4
6. Calibrator 5, 200 U/ml, ready for use		1 x 1.0 ml	CAL 5
7. Calibrator 6, 500 U/ml, ready for use		1 x 1.0 ml	CAL 6
8. Control 1, ready for use	green	1 x 1.0 ml	CONTROL 1
9. Control 2, ready for use	blue	1 x 1.0 ml	CONTROL 2
10. Antiserum polyclonal anti-alpha amylase antibody (rabbit), ready for use	blue	1 x 12 ml	ANTISERUM
11. Enzyme conjugate peroxidase-labelled alpha amylase, ready for use	orange	1 x 12 ml	ENZ | CONJ
12. Sample buffer, ready for use	light blue	1 x 100 ml	SAM | DIL
13. Wash buffer, 10x concentrate	colourless	1 x 100 ml	WASH | SOLN | 10x
14. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUB | TMB
15. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP | SOLN
16. Protective foil	---	3 pieces	
17. Test instruction	---	1 booklet	
18. Quality control certificate	---	1 protocol	

4. PREPARATION AND STABILITY OF THE REAGENTS

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antibodies can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.
- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.
- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.
- **Antiserum:** Ready for use. The antiserum must be mixed thoroughly before use.
- **Sample buffer:** Ready for use.
- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus
  9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working-strength wash buffer is stable for 4 weeks when stored at +2°C and +8°C and handled properly.
- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.
- **Stop solution:** Ready for use.

Warning: Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

5. PREPARATION AND STABILITY OF THE SAMPLES

Sample material: Human saliva (total saliva).
IBL-America recommends collecting saliva samples with blue cortisol Salivette® (Sarstedt AG & Co, Germany).

Stability: samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: samples are diluted **1:201** sample buffer.
For example: 5 µl sample to 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

6. INCUBATION

(Partly) manual test performance

Sample incubation: (1st step)

Transfer **20 µl of the calibrators, controls and diluted samples** into the individual microplate wells according to the pipetting protocol.

Pipette **100 µl of enzyme conjugate solution** (peroxidase-labelled alpha-amylase) into each of the microplate wells.

Pipette **100 µl of antiserum solution** (polyclonal anti-alpha amylase antibody) into each of the microplate wells.

Cover the microplate wells with the protective foil provided and incubate for **60 minutes** on a **microplate shaker (400 U/min)** at room temperature (+18 °C to +25 °C).

Washing:

Manual: Remove the protective foil, empty the wells and subsequently wash 3 times using 300 µl of working-strength wash buffer for each wash.
Automatic: Remove the protective foil and wash the reagent wells 3 times with 450 µl working-strength wash buffer (program setting: e.g. TECAN Columbus Washer “Overflow Mode”).

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction readings. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction readings.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Substrate incubation: (2nd step)

Pipette **100 µl of chromogen/substrate solution** into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C). Protect from direct sunlight.

Stopping:

Pipette **100 µl of stop solution** into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution.** Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices
Automated test performance using fully automated, open-system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 1**	**P 1**	**P 9**	**P 17**								
B	**C 2**	**P 2**	**P 10**	**P 18**								
C	**C 3**	**P 3**	**P 11**	**P 19**								
D	**C 4**	**P 4**	**P 12**	**P 20**								
E	**C 5**	**P 5**	**P 13**	**P 21**								
F	**C 6**	**P 6**	**P 14**	**P 22**								
G	**Co1**	**P 7**	**P 15**	**P 23**								
H	**Co2**	**P 8**	**P 16**	**P 24**								

The pipetting protocol for microplate strips 1 to 4 is an example for the analysis of 24 samples (P 1 to P 24).

The calibrators (C 1 to C 6), the controls 1+2 (Co1 + Co2), and the samples have each been incubated in one well. The reliability can be improved by duplicate determinations for each sample.
The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run.

7. RESULTS

The standard curve from which the alpha-amylase concentration in the unknown saliva samples can be taken is obtained by point-to-point plotting of the extinction readings measured for the 6 calibration sera against the corresponding units (linear/log). Use "4-parameter logistics" plotting for calculation of the standard curve by computer. For correct logarithmic representation it might be necessary to set the concentration of calibrator 1 to e.g. 0.1 U/ml.
If the extinction for a sample lies above the extinction of calibrator 6 (corresponding to 500 U/ml), the result should be reported as “>500 U/ml”. It is recommended that the sample be re-tested at a dilution of e.g. 1:401 instead of 1:201. The result in U/ml read from the calibration curve for this sample must then be multiplied by factor 2.
For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another IBL-America recommends retesting the samples.

8. TEST CHARACTERISTICS

Calibration: The standards and controls are calibrated gravimetrically.
For every group of tests performed, the readings of the concentrations must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature (+18°C to +25°C) during the incubation steps, the greater will be the extinction. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antibodies: A polyclonal anti-alpha amylase antibody is used, which specifically detects alpha amylase in human saliva.

Linearity: The linearity of the ELISA was determined by assaying at least 4 serial dilutions of 3 saliva samples. The linear regression was calculated and R^2 amounts to > 0.95 in all samples. The Alpha Amylase Saliva ELISA is linear at least in the tested concentration range (12 to 500 U/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable alpha amylase concentration. The detection limit of the Alpha Amylase Saliva ELISA is 3.6 U/ml.

Cross-reactivity: This ELISA specifically detects alpha amylase in human saliva. Cross-reactions with other amylases are listed in the table below:

Cross reactant	%
Alpha amylase in human saliva	100
Porcine pancreatic alpha amylase	< 0.23
Alpha amylase from Bacillus sp.	< 0.01

Interference: Contamination with blood up to a concentration of 4.0 % (v/v) did not cause interference with the ELISA. Red tint of the alpha-amylase sample indicates significant contamination with blood. The sample should not be used. We recommend taking a new sample at a later stage instead. Sodium azide can be added as preservative agent. A concentration of up to 0.9% has no effect on the measurement result.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 samples. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on duplicate determinations performed in 5 different runs.

Intra-assay precision, n = 20		
Saliva	**Mean value (U/ml)**	**CV (%)**
1	100	4.9
2	148	3.6
3	242	5.5

Inter-assay precision, n = 2 x 5		
Saliva	**Mean value (U/ml)**	**CV (%)**
1	23	9.6
2	105	4.2
3	234	4.8

Correlation: A comparison of the IBL-America assay with several reference tests yielded the following correlation values (sample range of 0-958 U/ml):

Salimeterics, Alpha amylase enzymatic test	**EI = 1.04 x Salimetrics – 1.56 U/ml** **n = 38; R^2 = 0.982**

SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Française	Espanol	Italiano
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	utilisation Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
V<x>	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta

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