

alpha-Amylase Saliva Assay

Enzymatic assay for the determination of alpha amylase activity
in human saliva.

REF **RE80111**

 **96**

   **2-8°C**

EU: **IVD** 



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1. INTENDED USE

Enzymatic assay for the determination of alpha amylase activity in human saliva.

2. SUMMARY AND EXPLANATION

The measurement of alpha Amylase activity in saliva is useful in the field of psychobiology of stress. A lot of studies have shown that the salivary alpha Amylase activity correlates with the Sympathoadrenal Medullary System activity.

Free salivary cortisol is an established non-invasive marker of hypothalamus pituitary adrenal (HPA) axis activity. Now salivary alpha Amylase seems to represent a such wellcharacterized salivary marker for the activity of the sympatho-adrenal medullar (SAM) system. Salivary alpha Amylase also seems to be related with chronic stress, posttraumatic stress disorder, behavior, cognitive function and health. Furthermore, salivary alpha Amylase increases can be elicited by SNS activating drugs (like yohimbine) and prevented by beta-blockade (like propranolol).

3. TEST PRINCIPLE

This assay is a liquid phase enzymatic assay. The salivary alpha Amylase metabolizes specifically the substrate. The intensity of the colour developed is proportional to the activity of alpha amylase in the sample. The results of samples can be determined directly by using the standard curve.

4. WARNINGS AND PRECAUTIONS

1. For *in-vitro diagnostic* use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. In case of severe damage of the kit package please contact IBL or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots. Do not use expired reagents.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details. Material Safety Data Sheets for this product are available on the IBL-Homepage or upon request directly from IBL.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according to national biohazard and safety guidelines or regulations.
8. The cleaning staff should be guided by the professionals regarding potential hazards and handling.
9. Some reagents contain sodium azide (NaN₃) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN₃ may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.


5. STORAGE AND STABILITY

The kit is shipped at ambient temperature and should be stored at 2-8 °C. Keep away from heat or direct sunlight. The storage and stability of specimens and prepared reagents is stated in the corresponding chapters.

6. SPECIMEN COLLECTION AND STORAGE**Saliva**

The patient should not eat, drink, chew gums or brush teeth for 30 min before sampling. Otherwise rinse mouth thoroughly with cold water 5 min prior to sample collection. Do not collect samples when oral diseases, inflammation or lesions exist (blood contamination).

Saliva can be collected in a suitable sampling device. A minimum of 0.5 mL liquid should be collected. Saliva flow can be stimulated by chewing on a piece of Parafilm®. It is recommended to freeze samples at -20°C prior to laboratory testing. After thawing, mix and centrifuge 10 min at 2000 – 3000 x g to remove particulate material.

	Take care that the saliva samples are visually okay. (Reddish color indicating blood contamination)		
Storage	18 – 25 °C	2 – 8 °C	≤ -20 °C (Aliquots)
Stability	1 day	28 days	≥ 6 months

7. MATERIALS SUPPLIED

Quantity	Symbol	Component
1 x 12 x 8	MTP	Microtiter Plate Break apart strips.
3 x 200 µL	CAL LYO	Stock standard , lyophilized Contains: human salivary alpha amylase. Reference method for the calibration: tempered photometer. Exact concentrations see vial labels or QC certificate.
2 x 3 x 200 µL	CONTROL 1 LYO CONTROL 2 LYO	Control 1+2 , lyophilized Contains: human salivary alpha amylase. Reference method for the calibration: tempered photometer. Exact concentrations see vial labels or QC certificate.
1 x 40 mL	DILBUF CONC	Sample Buffer, Concentrate (10x) Contains: PBS, BSA, stabilizers.
1 x 21 mL	SUBS	Substrat Solution Ready to use. Contains: CNPG3, stabilizers.

8. MATERIALS REQUIRED BUT NOT SUPPLIED

1. Micropipettes (Multipette Eppendorf or similar devices, < 3 % CV). Volume: 10; 100; 200; 1000 µL
2. Disposable tubes for sample dilution
3. 8-Channel Micropipettor with reagent reservoirs
4. Microtiter plate reader capable of reading absorbance at 405 nm (reference wavelength 600-650 nm)
5. Bidistilled or deionised water
6. Paper towels, pipette tips and timer
7. Vortex mixer

9. PROCEDURE NOTES

1. Any improper handling of samples or modification of the test procedure may influence the results. The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions. Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each component and specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. It is advised to determine samples in duplicate to be able to identify potential pipetting errors.
5. Use a pipetting scheme to verify an appropriate plate layout.
6. Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.
7. The alpha-amylase is found in sweat and in saliva. Please wear gloves during the assay and do not pipette with the mouth.

10. PRE-TEST SETUP INSTRUCTIONS**10.1. Preparation of concentrated components**

Component	Relation	Diluent	Remarks	Storage	Stability
Sample Buffer DILBUF CONC	1:10	bidist. water	e.g. 10 mL Sample Buffer + 90 mL bidist. water Mix without foaming.	2-8°C	4 weeks

10.2. Preparation of lyophilized components

Component	with	Diluent	Remarks	Storage	Stability
CAL LYO	200 µL	Diluted sample buffer	Let stand for 15 min. Mix without foaming.	2-8°C	7 days
CONTROL 1+2 LYO	200 µL			2-8°C	7 days

10.3. Preparation of the standards

The reconstituted stock standard has to be diluted to 1:301. This is the standard 1. Then a serial dilution of this solution permits to obtain standards at different concentrations. The following example shows how to obtain a standard curve with 5 calibration points.

Name	Relation	Diluent	Remarks	Calculation U/mL
Standard 1	1:301	Diluted sample buffer	10 µL reconstituted stock standard + 3 mL diluted sample buffer Mix well before further dilution.	See label e.g.: 400 U/mL
Standard 2	1:2	Diluted sample buffer	100 µL Standard 1 + 100 µL diluted sample buffer Mix well before further dilution.	e.g.: 200 U/mL
Standard 3	1:2	Diluted sample buffer	100 µL Standard 2 + 100 µL diluted sample buffer Mix well before further dilution.	e.g.: 100 U/mL
Standard 4	1:2	Diluted sample buffer	100 µL Standard 3 + 100 µL diluted sample buffer Mix well before further dilution.	e.g.: 50 U/mL
Standard 5	-	Diluted sample buffer	Only diluted sample buffer	0 U/mL

10.4. Predilution of Controls and Samples

The samples must be pre-diluted up to 1:301 as follows:

	Relation	Diluent	Remarks
Sample	1:301	Diluted sample buffer	e.g.: 10 µL Sample (Saliva) + 3 mL diluted sample buffer. Mix well after dilution!
Controls	1:301	Diluted sample buffer	e.g.: 10 µL Control (reconstituted) + 3 mL diluted sample buffer. Mix well after dilution!

Samples containing concentrations higher than the highest standard have to be diluted further.

11. TEST PROCEDURE

1.	Pipette 10 µL of each prediluted Standard , prediluted Controls and prediluted sample into the respective wells of the Microtiter Plate.
2.	Pipette 200 µL of the Substrate solution into each well. Shake the plate carefully.
3.	For adding of the Substrate solution use, if available, an 8-channel Micropipettor. Use positive displacement and avoid formation of air bubbles.
4.	Incubate 3 min at room temperature (18-25°C).
5.	Measurement 1: Measure the optical density (OD) with a photometer at 405 nm (Reference-wavelength: 600-690 nm).
6.	During the incubation the alpha Amylase present in the sample will metabolizes specifically the substrate. It is important to obtain a good differentiation between the standards. Nevertheless <u>the OD of highest standard obtained for the second measurement should not exceed 2.900.</u> Follow the development of the OD at different times to avoid overrun. This is most particularly appropriated in case of high room temperature. The results of alpha Amylase activity will not be affected!
7.	Incubate another 5 min (total incubation time of 8 min) at room temperature (18-25°C). In case of high room temperature or first use of the assay it is recommended to measure the plate additionally after 3 min (total incubation time of 6 min).
8.	Measurement 2: Measure the optical density (OD) with a photometer at 405 nm (Reference-wavelength: 600-690 nm).

12. QUALITY CONTROL

The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated. Each laboratory should use known samples as further controls.

In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

13. CALCULATION OF RESULTS

Calculate the **delta OD** for each standard, control and sample by deducting the OD of the "Measurement 1" from the OD of "Measurement 2".

The obtained **delta OD** of the standards (y-axis, linear) is plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 Parameter Logisitcs or Logit-Log.

For the calculation of the standard curve, apply each delta OD of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used).

The alpha amylase activity of the samples and controls can be read directly from the standard curve.

Results of samples of higher pre-dilution have to be multiplied with the dilution factor.

Samples showing concentrations above the highest standard have to be diluted as described in PRE-TEST SETUP INSTRUCTIONS and reassayed.

You may contact IBL for assistance in the calculation of results.

The results are expressed in U/mL. To convert in International System (SI) of Units please multiply with the factor 0.01667. The results will then be expressed in nKat/L.












14. PERFORMANCE

Precision	Mean Activity (U/mL)	SD (U/mL)	CV (%)	N
Intra-Assay	47.0 – 166	1.7 – 3.8	3.7 – 2.3	20
Inter-Assay	34.7 – 260	2.2 – 18.0	6.2 – 6.9	20
Linearity	Sample Saliva	Dilution	Measured Activity (U/mL)	Recovery (%)
	1	1:1	9.7	-
		1:1.5	9.8	101
		1:2.3	9.9	102
		1:3.4	9.8	101
		1:5.1	10.6	110
	2	1:1	50.5	-
		1:1.5	51.0	101
		1:2.3	51.1	101
		1:3.4	49.3	98
		1:5.1	48.6	96
	3	1:1	289.5	-
		1:1.5	284.6	98
		1:2.3	288.5	100
		1:3.4	287.6	99
1:5.1		292.6	101	
Recovery	Endogenous activity (U/mL)	Added Activity (U/mL)	Measured Activity (U/mL)	Recovery (%)
	Saliva 1 (25.5)	225.0	232.9	93
		112.5	124.8	90
		56.3	73.6	90
	Saliva 2 (38.9)	225.0	252.4	96
		112.5	452.5	101
		56.3	95.0	100
	Saliva 3 (107.1)	225.0	308.0	93
		112.5	200.4	91
		56.3	155.3	95
Method Comparison	IBL = 1.0198 x commercially available enzymatic assay – 5.0515			r = 0.997 n = 24

15. PRODUCT LITERATURE REFERENCES

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Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di evaluazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazemar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabbicante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

COMPLAINTS: Complaints may be submitted initially written or vocal. Subsequently they need to be filed including the test performance and results in writing in case of analytical reasons.

WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

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