SALIVARY URIC ACID

ENZYMATIC ASSAY KIT

For Research Use Only
Not for use in Diagnostic Procedures

Item No. 1-3802, (Single) 96-Well Kit;
1-3802-5, (5-Pack) 480 Wells

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Intended Use

The Salimetrics® Uric Acid Enzymatic Assay Kit is a colorimetric assay specifically designed and validated for the quantitative measurement of salivary Uric Acid. It is not intended for diagnostic use. It is intended only for research use in humans. Salimetrics has not validated this kit for serum or plasma samples.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in unreliable values.

For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

Uric Acid (UA) is an end product of purine nucleotide catabolism in humans that provides health benefits at normal levels, constituting a large portion of the antioxidant capacity of blood (1). In general, antioxidants are critical in minimizing the systemic physiological stress of free-radical oxygen species on the body preventing a condition known as Oxidative Stress. The cumulative damage caused by Oxidative Stress has been linked to a wide range of health problems including cancer, cardiovascular disease and age related neurodegenerative diseases among others (1-4). Therefore, the antioxidant properties of UA are thought to provide a protective role against these conditions. Consistent with this concept, a recent report indicates that high blood levels of UA may be protective against Alzheimer diseases (5).

Systemic UA concentrations are influenced by dietary levels of purine rich foods, body mass index (BMI), and cardiometabolic risk factors, such as high blood pressure, high density lipoprotein (HDL), triglyceride levels and fasting blood glucose (1,6). When UA concentrations are elevated in a condition known as hyperuricemia, significant harmful health effects result. Blood UA levels above 7 mg/dl leads to the formation of monosodium urate (MSU) crystals. After sustained hyperuricemia, these MSU crystals deposit in tendons and joints to cause severe diseases including gout, kidney stones and several forms of kidney disease. Gout is the most prevalent inflammatory arthritis worldwide and frequent monitoring of UA levels is critical for disease management (7). In the case of kidney stones, approximately 5-10% of the 3.3 million Americans seeking medical care for kidney stones are due to elevated UA. High blood UA levels are also associated with a wide variety of diseases including hypertension, increased cardiovascular mortality, obesity and metabolic syndrome (6).

Several studies have reported that a linear relationship exists between serum and salivary UA levels (8-11) and salivary UA may be a useful biomarker for monitoring metabolic syndrome that may help mitigate cardiometabolic risk (8).

Test Principle

This method utilizes a proprietary enzymatic reaction mixture that enables the detection of Uric Acid by the production of a red chromogen after a short incubation. This chromogen is quantitatively measured at a wavelength of 515 (or 520) nm. The amount of Uric Acid in the sample is directly proportional to the increase in absorbance at this wavelength. For ease of use, the reaction is read in a 96-well microtiter plate with standard and controls provided.

Safety Precautions

Read Safety Data Sheets before handling reagents.

Hazardous Ingredients

The standard and controls contain 0.05% sodium azide as a preservative. Do not ingest. Upon contact with acid, sodium azide forms toxic hydrazoic acid. Explosive metal azides may form in copper or lead plumbing. Disposal requires large volumes of water to prevent the buildup of azide.

We recommend the procedures listed below for all kit reagents.

Handling

Follow good laboratory practices when handling kit reagents. Laboratory coats, gloves, and safety goggles are recommended. Wipe up spills using appropriate absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing call a physician.

The above information is believed to be accurate but is not all-inclusive. This information should be used only as a guide. Salimetrics will not be liable for accidents or damage resulting from misuse of product.

Safety Data Sheets are available by contacting Salimetrics at support@salimetrics.com (See www.salimetrics.com for alternative contact options).

General Kit Use Advice

- This kit uses break-apart microtitre strips. You may run less than a full plate. Store unused wells in the bag and use in the frame provided.
- Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month. Store all reagents at 2-8°C.
- The quantity of reagent provided with a single kit is sufficient for three partial runs. The volume of Uric Acid Reagent prepared for assays using less than a full plate should be scaled down accordingly.
- Do not mix components from different lots of kits.
- Protect the Uric Acid Reagent solution from exposure to direct sunlight. ***Caution: Do not allow the reagent to come into contact with aluminum foil as it will catalyze the reagent and affect results.***
- To ensure highest quality assay results, pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate. Ideally, the process should be completed within 20 minutes or less.
- When using a multichannel pipette, Uric Acid Reagent solution should be added to wells at the same time, using the dispensing mode to avoid introducing bubbles into the wells.
- When running multiple plates, or multiple sets of strips, a standard, blank and controls must be run with each individual plate and/or set of strips.
- The temperature of the laboratory may affect assays. Salimetrics' kits have been validated at 68-74°F (20-23.3°C). Higher or lower temperatures may affect OD values.
- Routine calibration of pipettes and other equipment is critical for the best possible assay performance.
- When mixing plates during assay procedures, avoid speeds that spill the contents of the wells.

Storage

All unopened components of this kit are stable at 2-8°C until the kit's expiration date.

Specimen Collection

Avoid sample collection within 60 minutes after eating a major meal, drinking green tea, Vitamin C or Vitamin C rich foods or within 12 hours after consuming alcohol. Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected.

Collect whole saliva by unstimulated passive drool. Donors may tilt the head forward, allowing the saliva to pool on the floor of the mouth, then pass the saliva through the SalivaBio Collection Aid (SCA) into a polypropylene vial. Collection protocols/methods are available online at www.salimetrics.com or upon request.

Samples visibly contaminated with blood should be recollected. Samples may be screened for possible blood contamination (12,13) using our Blood Contamination EIA Kit (Item Nos. 1-1302/1-1302-5). Do not use dipsticks, which result in false positive values due to salivary enzymes.

Record the time and date of specimen collection.

Sample Handling and Preparation

After collection, it is important to keep samples cold in order to avoid bacterial growth in the specimen. Refrigerate sample within 30 minutes, and freeze at or below -20ºC within 4 hours of collection. (Samples may be stored at -20ºC for up to 6 months.) For long term storage, refer to the Salimetrics Collection and Handling Advice Booklet.

Do not add sodium azide to saliva samples as a preservative, as it may cause interference when testing samples for multiple analytes.

On day of assay, thaw the saliva samples completely, vortex, and centrifuge at 1500 x g for 15 minutes. Freezing saliva samples will precipitate mucins. Centrifuging removes mucins and other particulate matter which may interfere with the assay and affect results. Samples should be at room temperature before adding to assay plate. Pipette clear sample into appropriate wells. Re-freeze saliva samples as soon as possible after adding to the assay plate. Re-centrifuge saliva samples each time that they are thawed. Avoid multiple freeze-thaw cycles.

Materials Supplied with Single Kit

	Item	Quantity/Size
1	**Microtitre Plate** Break apart. Use number of strips desired.	1/96 well
2	**Uric Acid Standard** 5.0 mg/dL. Ready to use. Contains: Uric Acid, buffer, preservative.	1 vial / 200 µL
3	**Uric Acid Controls** High & Low. Ready to use. Contain: Uric Acid, buffer, preservative.	2 vials / 200 µL each
4	**Uric Acid Reagent** Ready-to-use. Contains: uricase, peroxidase, buffer, stabilizer.	1 bottle / 30 mL
5	**Uric Acid Sample Diluent** Contains: phosphate buffer, preservative.	1 bottle / 4 mL
6	**Adhesive Plate Covers**	2

Materials Needed But Not Supplied

- Precision pipette to deliver* 10 µL
- Precision multichannel pipette to deliver* 190 µL
- Vortex
- 37ºC Microplate incubator/shaker with 0.08-0.17 inch orbit capable of operating at 500 rpm
- Plate reader with a 515 (or 520) nm filter
- 37ºC Incubator
- Reagent reservoirs
- One disposable polypropylene tube to hold at least 20 mL
- Pipette tips
- Centrifuge capable of 1500 x g

  * without employing "blow-out" mechanism

Reagent Preparation

- Bring Uric Acid Reagent to 37ºC before use.
- Bring all other reagents to room temperature and mix before use. A minimum warm-up time of 30 minutes is recommended.

Procedure

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout. Here is a suggested layout. (Standards, controls, and saliva samples should be assayed in duplicate.)

	1	2	3	4	5	6	7	8	9	10	11	12
A	5.0 Std	5.0 Std										
B	Ctrl-H	Ctrl-H										
C	Ctrl-L	Ctrl-L										
D	Blank	Blank										
E	SMP-1	SMP-1										
F	SMP-2	SMP-2										
G	SMP-3	SMP-3										
H	SMP-4	SMP-4										

Step 2: Set your microplate incubator/shaker to 37ºC.

Step 3: Warm the Uric Acid Reagent to 37ºC. Be sure reagent has reached 37ºC before use.

Step 4: Keep the desired number of strips in the strip holder and place the remaining strips back in the bag.

Step 5:

- Pipette 10 µL of standards, controls, and saliva samples into appropriate wells.
- Pipette 10 µL of Uric Acid Sample Diluent into 2 wells to serve as the blank.

Step 6: Add 190 µL of preheated (37ºC) Uric Acid Reagent to each well simultaneously using a multichannel pipette. ***Do not return any of the Uric Acid Reagent left in the tips to the remaining stock once you have dispensed it into the wells. This could contaminate the stock contents and affect any subsequent testing.***

Step 7: Place adhesive cover provided over plate. Mix plate in a microplate incubator/shaker (***preheated to 37ºC***) continuously at 500 rpm for 10 minutes.

Step 8: Carefully remove plate from 37ºC microplate incubator/shaker. Let sit at room temperature for 10 minutes.

Step 9:

- Remove adhesive cover. Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read plate immediately in a plate reader at 515 (or 520) nm.

Quality Control

The Salimetrics' High and Low Uric Acid Controls should be run with each assay. The control ranges established at Salimetrics are to be used as a guide. Each laboratory should establish its own range. Variations between laboratories may be caused by differences in techniques and instrumentation.

Calculations

1. Compute the average optical density (OD) for the blank wells.
2. Subtract the average OD for the blank wells from the OD of the standard, controls, and saliva samples.
3. Compute the average blanked OD for the standard only.
4. Determine the concentrations of each control and saliva sample using the following formula:

$$\frac{\text{Blanked OD (control, saliva samples)}}{\text{Average Blanked OD (standard)}} \times \text{Standard Conc. (mg/dL)} = \text{Uric Acid Conc. (mg/dL)}$$

Example:

Blanked OD_{515} of saliva sample = 0.108

Average Blanked OD_{515} of the 5.0 mg/dL standard = 0.206

$$\frac{0.108}{0.206} \times 5\,\text{mg/dL} = 2.62\ \text{mg/dL}$$

5. Samples with Uric Acid values greater than 20 mg/dL should be diluted with Uric Acid Sample Diluent and rerun for accurate results. If a dilution of the sample is used, multiply the assay results by the dilution factor.

Standards, blanks and controls must be run with each full or partial plate.

Typical Results

The results shown below are for illustration only and should not be used to calculate results from another assay.

Well	Sample	Average OD	Average Blanked OD	Uric Acid (mg/dL)
A1,A2	Std	0.255	0.206	5
D1,D2	Blank	0.049	0	0

Limitations

- Linearity is established from 0 to 20 mg/dL.
- Samples with Uric Acid values greater than 20 mg/dL should be diluted with Uric Acid Sample Diluent and rerun for accurate results. To obtain the final Uric Acid concentration, multiply the concentration of the diluted sample by the dilution factor.
- See "Specimen Collection" recommendations to ensure proper collection of saliva specimens and to avoid interfering substances.
- Any quantitative results indicating abnormal Uric Acid levels should be followed by additional testing and evaluation.

Salivary Uric Acid Example Ranges*

Group	N	Range (mg/dL)	Median (mg/dL)	Q1 (mg/dL)	Q3 (mg/dL)
Adult	344	<0.07 – 12.68	3.02	1.76	4.21

*To be used as a guide only. Each laboratory should establish its own range.

Salivary Uric Acid Enzymatic Assay Kit Performance Characteristics

Precision

The intra-assay precision was determined from the mean of 20 replicates each.

Saliva Sample	N	Mean (mg/dL)	Standard Deviation (mg/dL)	Coefficient of Variation (%)
1	20	1.05	0.03	2.5
2	20	2.53	0.05	1.8
3	20	4.87	0.08	1.6
4	20	7.57	0.13	1.7
5	20	14.83	0.27	1.8

The inter-assay precision was determined from the mean of average duplicates for 24 separate runs.

Saliva Sample	N	Mean (mg/dL)	Standard Deviation (mg/dL)	Coefficient of Variation (%)
1	48	1.02	0.07	6.8
2	48	2.50	0.10	4.1
3	48	4.59	0.18	4.0
4	48	6.67	0.24	3.5
5	48	12.85	0.50	3.9

Recovery

4 saliva samples containing different levels of an endogenous Uric Acid were spiked with known quantities of Uric Acid and assayed.

Saliva Sample	Endogenous (mg/dL)	Added (mg/dL)	Expected (mg/dL)	Observed (mg/dL)	Recovery (%)
1	0.95	4.79	5.74	5.95	103.7
	0.95	2.43	3.38	3.51	103.6
	0.95	0.97	1.92	1.94	100.8
2	2.48	4.79	7.26	7.27	100.1
	2.48	2.43	4.91	4.59	93.6
	2.48	0.97	3.45	3.27	94.7
3	4.10	4.79	8.88	8.31	93.5
	4.10	2.43	6.53	6.10	93.4
	4.10	0.97	5.07	4.68	92.3
4	8.62	4.79	13.40	12.31	91.8
	8.62	2.43	11.05	10.07	91.2
	8.62	0.97	9.59	8.87	92.4

Sensitivity

Analytical Sensitivity

The lower limit of sensitivity was determined by interpolating the mean optical density plus 2 SDs of 10 sets of duplicates at the 0 mg/dL level. The minimal concentration of Uric Acid that can be distinguished from 0 is 0.07mg/dL.

Functional Sensitivity

The limit of quantification was determined as the concentration of saliva having low endogenous Uric Acid diluted with charcoal stripped saliva, tested as 20 replicates each run with 6 independent runs, resulting in a CV of 18.72%. The functional sensitivity of the Salivary Uric Acid Enzymatic Assay is 0.26 mg/dL.

Sample Dilution Recovery

Three samples were serially diluted with Uric Acid Sample Diluent and assayed.

Saliva Sample	Dilution Factor	Expected (mg/dL)	Observed (mg/dL)	Recovery (%)
1	undiluted	N/A	2.55	N/A
	1:2	1.28	1.26	98.7
	1:4	0.64	0.62	97.3
	1:8	0.32	0.32	101.4
2	undiluted	N/A	4.84	N/A
	1:2	2.42	2.54	105.1
	1:4	1.21	1.26	104.4
	1:8	0.61	0.65	107.6
3	undiluted	N/A	8.88	N/A
	1:2	4.44	4.66	104.9
	1:4	2.22	2.34	105.4
	1:8	1.11	1.19	106.9

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Seller's Limited Warranty

"Seller warrants that all goods sold hereunder will be free from defects in material and workmanship. Upon prompt notice by Buyer of any claimed defect, which notice must be sent within thirty (30) days from date such defect is first discovered and within three months from the date of shipment, Seller shall, at its option, either repair or replace the product that is proved to Seller's satisfaction to be defective. All claims should be submitted in written form. This warranty does not cover any damage due to accident, misuse, negligence, or abnormal use. Liability, in all cases, will be limited to the purchased cost of the kit.

It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties."

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