ab65344

Uric Acid Assay Kit (Colorimetric/Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of uric acid in various samples.

This product is for research use only and is not intended for diagnostic use.

Version 7a Last Updated 19 June 2025

Table of Contents

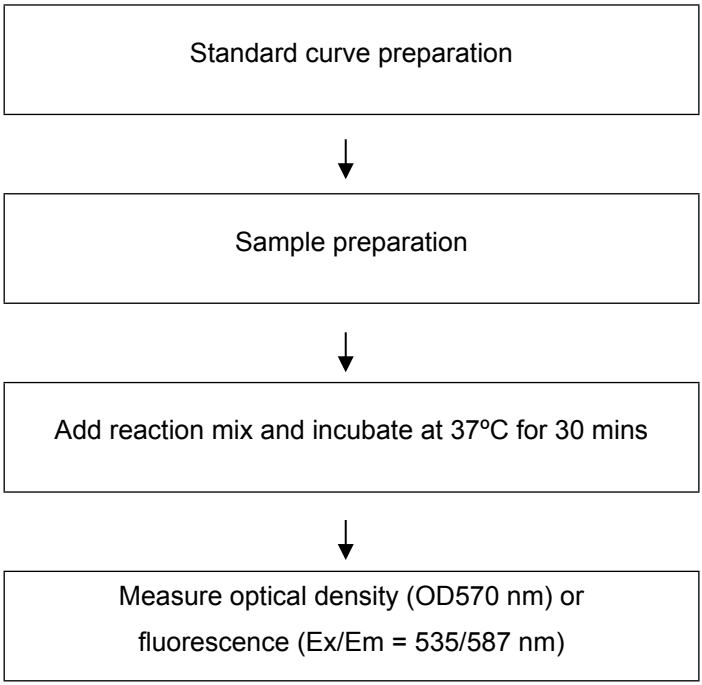
1. BACKGROUND

Uric Acid Assay Kit (colorimetric/fluorometric) (ab65344) provides a convenient means for detecting uric acid in biological samples such as serum and urine. Pretreatment of samples are not required. Uric acid level can be measured using fluorometric (at Ex/Em = 535 / 587 nm) or colorimetric (at λ= 570 nm) methods.

Uric acid in serum is the end product of purine metabolism, and is cleared through the kidney by glomerular filtration. However, human often lacks the necessary enzyme called urate oxidase (uricase), and therefore abnormal uric acid may be accumulated in blood.

Recent evidences show the close association between serum urate level and cardiovascular morbidity and mortality, especially among persons at high cardiovascular risk, including those with hypertension, diabetes and congestion heart failure.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 2	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Uric Acid Enzyme Mix	1 vial	-20°C	-20°C
Uric Acid Standard	1 mL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 2 was previously labelled as Assay Buffer II and Uric Acid Assay Buffer, and OxiRed™ Probe as OxiRed Probe and Uric Acid Probe (in DMSO, anhydrous). The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Colorimetric or fluorescent microplate reader – equipped with filter for OD570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: black plates (clear bottoms) for fluorometric assay; clear plates for colorimetric assay
- Microcentrifuge
- Pipettes and pipette tips
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 2:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **OxiRed™ Probe:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Store at -20°C protected from light and moisture. Once the OxiRed™ Probe is thawed, use with two months.

9.3 **Uric Acid Enzyme Mix:**

Dissolve in 220 µL uric acid Assay Buffer 2. Pipette up and down to dissolve it completely. Aliquot standard so that you have enough to perform the desired number of assays. Use within two months. Store at -20°C. Keep on ice while in use.

9.4 **Uric Acid Standard:**

Ready to use as supplied. Equilibrate to room temperature before use. If precipitation is noticed, centrifuge and take supernatant. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 For the colorimetric assay:

10.1.1 Using uric acid standard (2 nmol/µL), prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer 2 (µL)	Final volume standard in well (µL)	End [Uric Acid] in well
1	0	150	50	0 nmol/well
2	12	138	50	8 nmol/well
3	24	126	50	16 nmol/well
4	36	114	50	24 nmol/well
5	48	102	50	32 nmol/well
6	60	90	50	40 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

10.2 For the flurometric assay:

10.2.1 Prepare a 0.2 nmol/µL uric acid standard by diluting 20 µL of 2 nmol/µL uric acid standard into 180 µL of Assay Buffer 2.

10.2.2 Using 0.2 nmol/µL uric acid standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer 2 (µL)	Final volume standard in well (µL)	End [Uric Acid] in well
1	0	150	50	0 nmol/well
2	12	138	50	0.8 nmol/well
3	24	126	50	1.6 nmol/well
4	36	114	50	2.4 nmol/well
5	48	102	50	3.2 nmol/well
6	60	90	50	4.0 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation 1-2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of Assay Buffer 2.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Centrifuge 2 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.6 Collect supernatant and transfer to a clean tube.

11.1.7 Keep on ice.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 500-1,000 µL (or ~ 4 - 6 volumes) of the Assay Buffer 2, on ice.

11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.5 Centrifuge 2 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.3 **Serum and Urine:**

Serum samples can be tested directly. When testing serum, we suggest using 2 – 20 µL/assay, diluted in Assay Buffer 2. Normal serum contains ~ 0.3 nmol/ µL uric acid. For urine samples, spin down to remove any suspended particulate matter before testing.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 2).
- Background wells = 50 µL Assay Buffer 2.

12.2 **Reaction Mix:**

Prepare Reaction Mix for each reaction

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
Assay Buffer 2	**46**	**47.6**
OxiRed™ Probe*	**2**	**0.4**
Uric Acid Enzyme Mix	**2**	**2**

**For fluorometric readings, using 0.4 µL/well of the OxiRed™ Probe decreases the background readings, therefore increasing detection sensitivity.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

12.3 Add 50 µL of Reaction Mix to each sample, standard and background wells.

12.4 Incubate at 37ºC for 30 minutes protected from light.

12.5 Measure output on a microplate reader.

- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Uric Acid.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$A = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

13.6 Concentration (nmol/mL) of uric acid in the test samples is calculated as:

$$Uric\ Acid\ Concentration = \frac{A}{V} X\ 1000$$

Where:

A = uric acid amount from the sample well in nmol.

V = sample volume added into the sample well (in µL).

Uric acid molecular weight is 168 g/mol.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

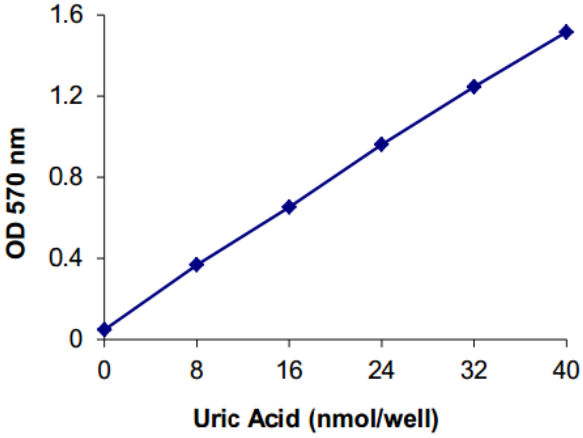


Figure 1: Typical uric acid standard calibration curve using colorimetric reading.

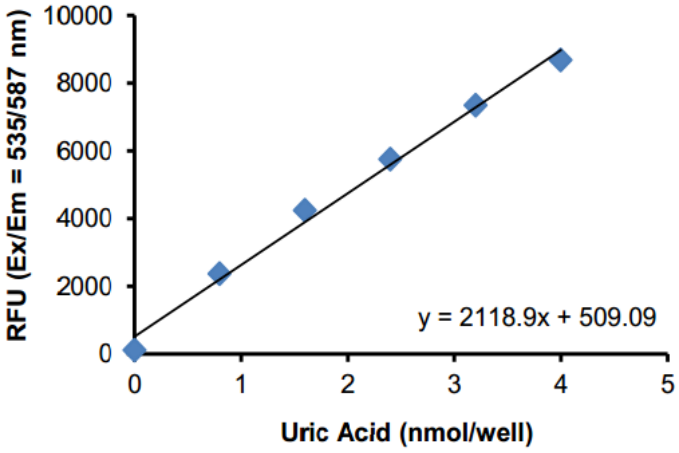


Figure 2. Typical uric acid standard calibration curve using fluorometric reading.

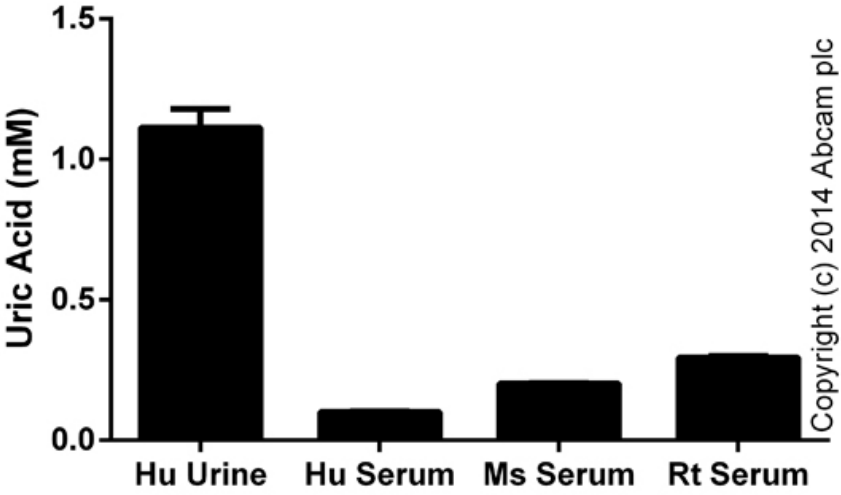


Figure 3: Uric acid measured in biological samples.

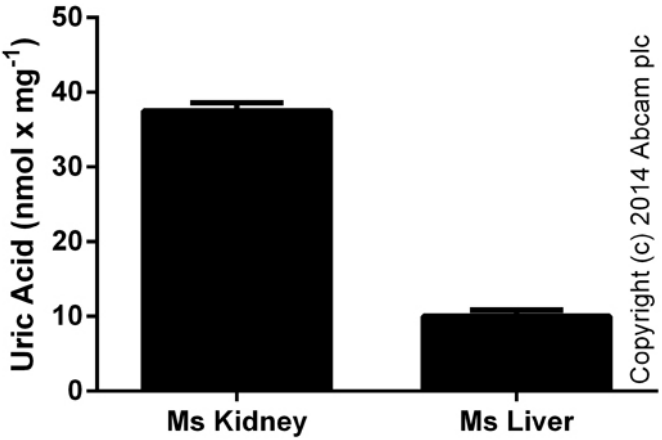


Figure 4: Uric acid measured in mouse tissue lysates, showing quantity (nmol) per mg of extracted protein.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, OxiRed™ Probe and enzyme mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Prepare Reaction Mix (Number samples + standards + 1).

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
Assay Buffer 2	**46**	**47.6**
OxiRed™ Probe	**2**	**0.4**
Uric Acid Enzyme Mix	**2**	**2**

- Set up plate for standard (50 µL), samples (50 µL).
- Add 50 µL Reaction Mix to each well.
- Incubate plate at 37ºC for 30 mins protected from light.
- Measure plate at OD570 nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

The OD values are very low. What could be the problem?

The most common reason for low OD values is that the assay buffer was cold, which led to a slow reaction.

What is the principle behind using the colorimetric versus the fluorometric assay?

The fluorometric assay is at least 10 times more sensitive than the colorimetric assay. The fluorometric method can be used for 0-5 nmol of uric acid. The colorimetric method can be used for >5 up to 40 nmol of uric acid.

18. **INTERFERENCES**

19. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)