Version 4a, Last updated 6 June 2025

ab234042 Uricase Assay Kit (Fluorometric)

For the measurement of Uricase activity in various biological samples/preparations.

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

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1. Overview

Uricase Assay Kit (Fluorometric) (ab234042) provides a quick and easy method for the measurement of uricase activity in a wide variety of samples.

In this assay, uricase oxidizes uric acid forming a product that reacts with the probe, thus generating a fluorescence signal (Ex/Em= 535/587nm). The generated fluorescence signal is directly proportional to the level of uricase activity in the sample. The kit provides a sensitive, high throughput and adaptable assay that can measure uricase activity as low as 10 µU in biological samples.

Uricase (factor-independent urate hydroxylase, urate oxidase (UO); EC 1.7.3.3) is an enzyme involved in the uric acid metabolism. Uric acid is the end product of purine metabolism, and high levels of uric acid in blood causes gout. Uricase is present in a wide range of mammals but absent from human beings. It has been widely used for the measurement of uric acid concentrations in biological samples and recombinant uricase (rasburicase) has been used as a drug for the prevention of tumor lysis syndrome.

Prepare samples, standards and blanks as directed and add to wells.

Make volume to 20 µl

Add 80 µL Reaction Mix and Background Reaction Mix to appropriate wells.

Immediately measure fluorescence (Ex/Em = 535/587) in kinetic mode at 30°C.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Uricase Assay Buffer	50 ml	-20°C	-20°C
Uricase Substrate	10 ml	-20°C	-20°C
OxiRed™ Probe	0.2 ml	-20°C	-20°C
Developer Solution V	1 vial	-20°C	-20°C
H_2O_2 Standard	100 µl	-20°C	-20°C
Uricase Positive Control	1 vial	-20°C	-20°C

PLEASE NOTE: Developer Solution V was previously labelled as Uricase Enzyme Mix, and OxiRed™ Probe as Uricase Probe (in DMSO). The composition has not changed.

3. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)
- Saturated ammonium sulfate solution (for mammalian tissue samples)
- Liquid nitrogen (for plant samples)
- (Optional) Protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail. Include if proteolytic degradation is suspected to be affecting uricase activity.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Uricase Assay Buffer

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Store at +4°C or -20°C.

5.2 Uricase Substrate

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Aliquot and store at -20°C.
4. Vortex before use.

5.3 OxiRed™ Probe (In DMSO)

1. Ready to use as supplied.
2. Warm to room temperature before use.
3. Store at -20°C.
4. Protect from light.

5.4 Developer Solution V

1. Reconstitute with 220 µl of Uricase Assay Buffer. Pipette up down gently to dissolve.
2. Aliquot and store at-20°C. Avoid freeze-thaw cycles.
3. Keep on ice whilst in use.

5.5 H_2O_2 Standard

1. Ready to use as supplied.
2. Keep on ice whilst in use.
3. Store at -20°C.

5.6 Uricase Positive Control

1. Reconstitute with 1 ml of Uricase Assay Buffer. Pipette up and down gently to dissolve.
2. Aliquot and store at -20°C. Avoid freeze-thaw cycles.
3. Keep on ice whilst in use.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Add 10 µl of the H_2O_2 Standard to 870 µl of dH_2O to generate 10 mM H_2O_2 Standard.
2. Further dilute the 10 mM H_2O_2 Standard by adding 10 µl of the 10 mM H_2O_2 standard into 990 µl dH_2O to make a 0.1 mM H_2O_2 Standard. Mix well.
3. Using 0.1 mM H_2O_2 standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	H_2O_2 Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount of H_2O_2 standard in well (nmol/well)
1	0	40	20	0
2	4	36	20	0.2
3	8	32	20	0.4
4	12	28	20	0.6
5	16	24	20	0.8
6	20	20	20	1.0

Each dilution has enough standard to set up duplicate readings (2 x 20 µL).

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

7.1 Plant Tissue:

1. Grind tissue samples in liquid nitrogen.
2. Rapidly homogenize tissue (~25 mg) with 100 µl ice cold Uricase Assay Buffer, and keep on ice for 10 minutes.
3. Centrifuge at 16,000 x *g* for 10 minutes at 4°C to remove cell debris and collect the supernatant.
4. Small molecules can interfere with the uricase activity, ultrafilter the samples with 10 kDa Spin Columns (ab93349) at 16,000 x *g* for 15 minutes at 4°C.
5. Collect the retentate and bring the volume of the retentate back to the original sample volume.
6. Add 2-10 µl of samples to designated wells of a 96-well clear plate.

7.2 Mammalian Tissue:

1. Rapidly homogenize tissue (~20 mg) with 100 µl ice cold Uricase Assay Buffer, and keep on ice for 10 minutes.
2. Centrifuge at 16,000 x *g* for 10 minutes at 4°C to remove cell debris and collect the supernatant.
3. Add 80 µl saturated ammonium sulfate solution per 100 µl lysate. Keep on ice for 30 minutes.
4. Centrifuge at 16,000 x *g* for 10 minutes at 4°C and discard the supernatant.
5. Resuspend the precipitated protein with Uricase Assay Buffer in the original volume of the lysate used.
6. Dilute the samples 5-fold and add 2-10 µl of diluted samples to designated wells of a 96-well clear plate.

Δ Note: *For all samples, prepare Sample Background Control wells by adding the same amount of samples in parallel wells. Adjust the volume to 20 µl with Uricase Assay Buffer.*

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Positive Control

1. Add 10 µl of the reconstituted Uricase Positive Control.
2. Adjust the volume to 20 µl/well with Uricase Assay Buffer.

8.2 Reaction wells set up:

- Standard wells = 20 µl standard dilutions.
- Sample wells = 2-10 µl samples (adjust volume to 20 µl/well with Uricase Assay Buffer).
- Sample Background Control wells = 2-10 µl samples (adjust volume to 20 µl/well with Uricase Assay Buffer).

8.3 Reaction mix:

1. Mix enough reagents for the number of assays to be performed.
2. Prepare a 5-fold dilution of OxiRed™ Probe (i.e. mix 20 µl of OxiRed™ Probe with 80 µl Uricase Assay Buffer).
3. Prepare 80 µl of Reaction Mix Background Mix for each well. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µl)	Background Reaction Mix (µl)
Uricase Assay Buffer	-	76 µl
Uricase Substrate	76 µl	-
Diluted OxiRed™ Probe	2 µl	2 µl
Developer Solution V	2 µl	2 µl

4. Add 80 µl of Reaction Mix into each standard and sample well.
5. Add 80 µl of Background Reaction Mix into the background control sample wells to initiate the reaction.

Δ Note: *Do not store Diluted OxiRed™ Probe. Prepare fresh dilutions as needed.*

8.4 Measurement:

Measure fluorescence (Ex/Em=535/587nm) immediately in a microplate reader in kinetic mode for 30-45 minutes at 30 °C.

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the Uricase activity found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. Subtract the mean value of the blank (zero standard) from all standards, controls and sample readings. This is the corrected absorbance.
3. Plot the corrected values for each standard as a function of the amount of H_2O_2 to generate a standard curve.
4. If significant, subtract the sample background control from its paired sample readings.
5. Select the linear portion of the kinetic curve for uricase activity calculation.
6. Apply Sample ΔRFU ($RFU_2 - RFU_1$) to the Standard Curve to get B nmol of product generated during the reaction time ($\Delta t = t_2 - t_1$).

$$\text{Sample Uricase Activity} = \frac{B}{(t * V)} * D \quad \textit{nmol/min/ml (mU/ml)}$$

Where:

B = amount of H_2O_2 generated in the sample well calculated from standard curve in nmol.

t = reaction time (minutes)

V = sample volume added in the sample wells (ml).

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

The specific activity in biological samples can be expressed as U/mg of protein.

Unit definition: One unit of uricase is the amount of enzyme that generates 1.0 µmol of H_2O_2 per minute at pH 7.5 at 30°C.

10. Typical Data

Data provided **for demonstration purposes only.**

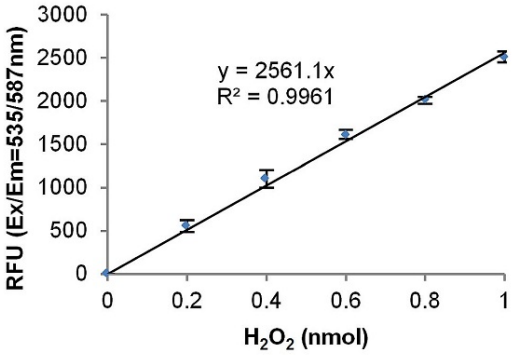

Figure 1. Uricase assay example standard curve.

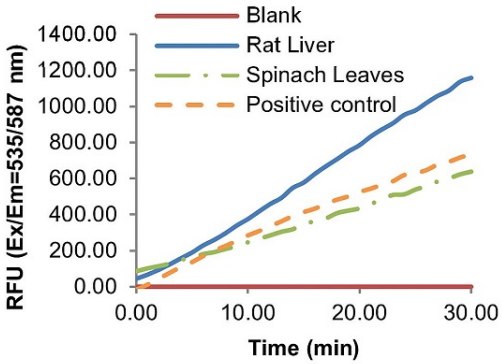

Figure 2. Uricase activity in rat liver (5.57 µg protein), spinach leaves (31.5 µg protein) and Uricase Positive Control.

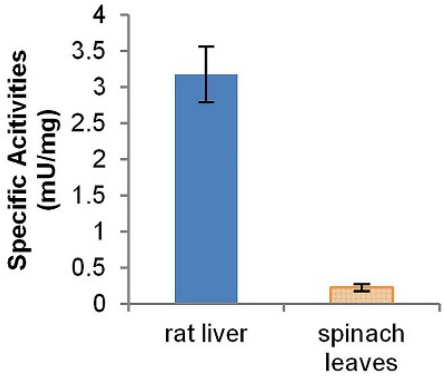

Figure 3: Uricase specific activity in rat liver and spinach leaves.

11. 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)