molecular probes by life technologies

Amplex® Red Uric Acid/Uricase Assay Kit

Catalog no. A22181

Table 1 Contents and storage

Material	A22181	Concentration	Storage*
Amplex® Red reagent (MW = 257, Component A)	2 vials of 0.26 mg	—	• –20˚C • Desiccate • Protect from light
Dimethylsulfoxide (DMSO), anhydrous (Component B)	500 µL	—	
Horseradish peroxydase (Component C)	20 U**	—	
Hydrogen peroxyde (H_2O_2) (MW = 34, Component D)	500 µL	~3%†	
5X Reaction Buffer (Tris-HCl, pH 7.5; Component E)	10 mL	0.5 M	
Uricase (Component F)	10 U‡	—	
Uric acid (MW = 168, Component G)	3 vials of 1.5 mL	5 mM in 1X Reaction Buffer	

*These storage conditions are appropriate when storing the entire kit upon receipt. For optimal storage of each component, see vial labels. When stored as directed this product is stable for 6 months. **One unit is defined as the amount of enzyme that will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 and 20˚C. †The actual concentration is indicated on the component label. ‡One unit is define as the amount of enzyme that will convert 1.0 µmole of uric acid to allantoin per minute at pH 8.5 and 25˚C

Approximate fluorescence excitation/emission maxima of reaction product: 571/585 in nm.

Introduction

The Amplex® Red Uric Acid/Uricase Assay Kit provides an ultrasensitive method for detecting uric acid or for monitoring uricase activity. In the assay, uricase catalyzes the conversion of uric acid to allantoin, hydrogen peroxide (H_2O_2) and carbon dioxide. The H_2O_2 then, in the presence of horseradish peroxidase (HRP), reacts stoichiometrically with Amplex® Red reagent to generate the red-fluorescent oxidation product, resorufin.[1] Resorufin has absorption and fluorescence emission maxima of approximately 571 nm and 585 nm, respectively (Figure 1), and because the extinction coefficient is high (54,000 $cm^{-1}M^{-1}$), the assay can be performed either fluorometrically or spectrophotometrically.

Serum uric acid is the end product of purine metabolism in the body tissues and is cleared through the kidneys by glomerular filtration. Most animals can metabolize uric acid to more readily excreted products, but humans lack the necessary enzyme, urate oxidase (uricase), as a result of the presence of two "nonsense mutations" in the human gene for uricase.[2] Increased uric acid levels may result from leukemia, polycythemia, ingestion of foods high in nucleoproteins (e.g. liver and kidney) or impaired renal function. Gout results from the deposit of uric acid in body joints.[3] Previous literature reports colormetric detection limits at 3.6 µM,[4] whereas the Amplex® Red Uric Acid/Uricase Assay Kit can be used to detect as little as 100 nM of uric acid in a purified system (Figure 2). The kit can also be used to detect a little as 0.2 mU/mL uricase in a purified system (Figure 3).

Figure 1 Normalized absorption and fluorescence emission spectra of resorufin, the product of the Amplex® Red reagent.

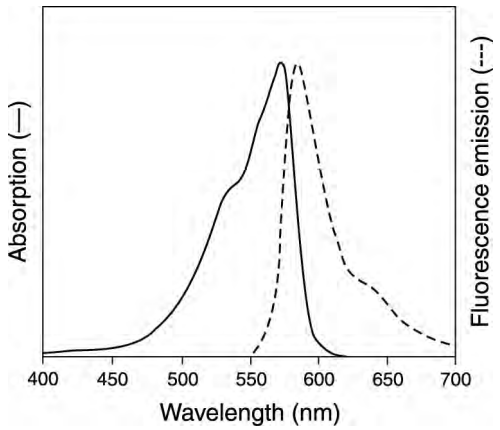


Figure 2 Detection of uric acid using the Amplex® Red reagen-based assay. Each reaction contained 50 µM Amplex® Red reagent, 0.2 U/mL HRP, 0.2 U/mL uricase, and the indicated amount of uric acid in 1X Reaction Buffer. After 30 minutes incubation at 37°C, fluorescence was measured in a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ±17.5 nm. A background fluorescence of 26 fluorescence units was substracted from each data point.

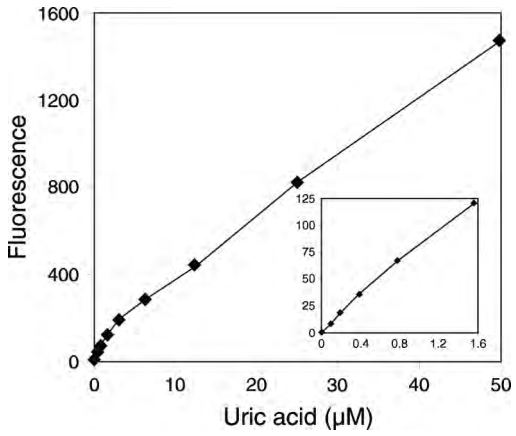
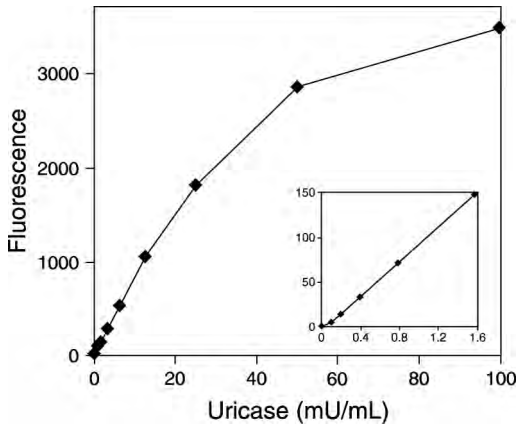


Figure 3 Detection of uric acid using the Amplex® Red reagen-based assay. Each reaction contained 50 µM Amplex® Red reagent, 0.2 U/mL HRP, 1 mM uric acid, and the indicated amount of uricase in 1X Reaction Buffer. After 30 minutes incubation at 37°C, fluorescence was measured in a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ±17.5 nm. A background fluorescence of 26 fluorescence units was substracted from each data point.



Before Starting

Storage and Handling

Upon receipt, the kit should be stored frozen at –20°C, protected from light. Stored properly, the kit components should remain stable for at least six months. Allow reagents to warm to room temperature before opening vials. The Amplex® Red reagent is somewhat air sensitive. Once a vial of Amplex® Red reagent is opened, the reagent should be used promptly. PROTECT THE Amplex® RED REAGENT FROM LIGHT.

Preparing Stock Solutions

1.1 Prepare a 10 mM stock solution of Amplex® Red reagent: Allow one vial of Amplex® Red reagent (Component A) and DMSO (Component B) to warm to room temperature. Just prior to use, dissolve the contents of the vial of Amplex® Red reagent (0.26 mg) in 100 µL of DMSO. Each vial of Amplex® Red reagent is sufficient for approximately 200 assays, with a final reaction volume of 100 µL per assay. This stock solution should be stored frozen at –20°C, protected from light.

1.2 Prepare a 1X working solution of Reaction Buffer by adding 4 mL of 5X Reaction Buffer stock solution (Component E) to 16 mL of deionized water (dH_2O). This 20 mL volume of 1X Reaction Buffer is sufficient for approximately 100 assays of 100 µL each with a 10 mL excess for making stock solutions and dilutions.

1.3 Prepare a 100 U/mL stock solution of horseradish peroxidase (HRP) by dissolving the contents of the vial of HRP (Component C) in 200 µL of 1X Reaction Buffer. After use, the remaining solution should be divided into small aliquots and stored frozen at –20°C.

1.4 Prepare a 20 mM H_2O_2 working solution by diluting the ~3% H_2O_2 stock solution (Component D) into the appropriate volume of dH_2O. The actual H_2O_2 concentration is indicated on the component label. For instance, a 20 mM H_2O_2 working solution can be prepared from a 3.0% H_2O_2 stock solution by diluting 23 µL of 3.0% H_2O_2 into 977 µL of dH_2O. Please note that although the ~3% H_2O_2 stock solution has been stabilized to slow degradation, the 20 mM H_2O_2 working solution will be less stable and should be used promptly.

1.5 Prepare a 100 U/mL uricase stock solution by dissolving the contents of the vial of uricase (Component F) in 100 µL of dH_2O. After use the remaining solution should be stored at –20°C.

The following procedure is designed for use with a fluorescence or absorbance multiwell plate scanner. For use with a standard fluorometer or spectrophotometer, volumes must be increased accordingly.

Please note that resorufin, the product of the Amplex® Red reaction, is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. For this reason, the final DTT or 2-mercaptoethanol concentration in the reaction should be no higher than 10 µM.

The absorption and fluorescence of resorufin are pH-dependent. Below the pK_a (~6.0), the absorption maximum shifts to ~480 nm and the fluorescence quantum yield is markedly lower. In addition, the Amplex® Red reagent is unstable at high pH (>8.5). For these reasons, the reactions should be performed at pH 7–8. We recommend using the included Reaction Buffer (pH 7.5) for optimal performance of the Amplex® Red reagent.

Uric Acid Assay

The following protocol describes the assay of uric acid in a total volume of 100 µL per microplate well. The volumes recommended here are sufficient for ~100 assays. The kit provides sufficient material for ~400 assays.

2.1 Prepare a uric acid standard curve by diluting the appropriate amount of 5 mM uric acid (Component G) into 1X Reaction Buffer to produce uric acid concentrations of 0 to 100 µM. Use 1X Reaction Buffer without uric acid as a negative control. A volume of 50 µL will be used for each reaction. Note that the final concentration will be twofold lower in the final reaction.

2.2 If desired, prepare a positive control by diluting the 20 mM H_2O_2 working solution (prepared in Step 1.4) to 10 µM in 1X Reaction Buffer.

2.3 Dilute the uric acid–containing samples in 1X Reaction Buffer. A volume of 50 µL will be used for each reaction.

2.4 Pipet 50 µL of the diluted samples, standards and controls into separate wells of a microplate.

2.5 Prepare a working solution of 100 µM Amplex® Red reagent containing 0.4 U/mL HRP and 0.4 U/mL uricase according to Table 2.

Table 2 Amplex® Red reagent working solution for uricase assay

Reaction Components	Amount needed (for ~100 assays)
Amplex® Red reagent stock solution (prepared in Step 1.1)	50 µL
HRP stock solution (prepared in Step 1.3)	20 µL
Uricase stock solution (prepared in Step 1.5)	20 µL
1X Reaction Buffer	4.91 mL
Total volume	5 mL

Note that the final concentration of each component will be two-fold lower in the final reaction.

2.6 Begin the reactions by adding 50 µL of the Amplex® Red reagent/HRP/uricase working solution to each microplate well containing the samples and controls.

2.7 Incubate the reactions for 30 minutes or longer at 37°C, protected from light. Because the assay is continuous (not terminated), fluorescence or absorbance may be measured at multiple time points to follow the kinetics of the reactions.

2.8 Measure the fluorescence or absorbance in a microplate reader using excitation in the range of 530–560 nm and emission detection at ~590 nm or absorbance at ~560 nm (see Figure 1).

2.9 For each point, correct for background fluorescence or absorbance by subtracting the value derived from the no-uric acid control.

Uric Acid Assay

The following protocol describes the assay of uricase activity in a total volume of 100 µL per microplate well. The volumes recommended here are sufficient for ~100 assays. The kit provides sufficient material for ~400 assays.

3.1 Prepare a uricase standard curve by diluting the 100 U/mL uricase stock solution (prepared in step 1.5) in 1X Reaction Buffer to produce uricase concentrations of 0 to 100 mU/mL. Use 1X Reaction Buffer without uricase as a negative control. A volume of 50 µL will be used for each reaction. Note that the uricase concentration will be twofold lower in the final reaction volume.

3.2 Dilute the uricase-containing samples in 1X Reaction Buffer. A volume of 50 µL will be used for each reaction.

3.3 If desired, prepare a positive control by diluting the 20 mM H_2O_2 work solution (prepared in step 1.4) to 10 µM in 1X Reaction Buffer.

3.4 Pipet 50 µL of the diluted uricase-containing standards, controls and samples into separate wells of a microplate.

3.5 Prepare a working solution of 100 µM Amplex® Red reagent containing 0.4 U/mL HRP and 1.0 mM uric acid according to Table 3.

Table 3 Amplex® Red reagent working solution for uric acid assays

Reaction Components	Amount needed (for ~100 assays)
Amplex® Red reagent stock solution (prepared in Step 1.1)	50 µL
HRP stock solution (prepared in Step 1.3)	20 µL
5 mM uric acid stock solution (Component G)	1 mL
1X Reaction Buffer	3.93 mL
Total volume	5 mL

Note that the final concentration of each component will be two-fold lower in the final reaction.

3.6 Begin the reaction by adding 50 µL of the Amplex® Red reagent/HRP/uric acid working solution to each microplate well containing the samples and controls.

3.7 Incubate the reaction for 30 minutes or longer at 37°C, protected from light. Because the assay is continuous (not terminated), fluorescence or absorbance may be measured at multiple time points to follow the kinetics of the reactions.

3.8 Measure the fluorescence or absorbance in a microplate reader using excitation in the range of 530–560 nm and emission detection at ~590 nm (see Figure 1).

3.9 For each point, correct for background fluorescence or absorbance by subtracting the value derived from the no-uricase control.

References

1. J Immunol Methods 202, 133 (1997); **2.** J Mol Evol 34, 78 (1992); **3.** Anal Chem 71, 1928 (1999); **4.** Clin Biochem 27, 93 (1994).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
A12222	Amplex® Red reagent (10-acetyl-3,7-dihydroxyphenoxazine)	5 mg
A22177	Amplex® Red reagent*packaged for high-throughput screening*	10 x 10 mg
A22181	Amplex® Red Uric Acid/Uricase Assay Kit *400 assays*	1 kit
A36006	Amplex® UltraRed reagent	5 x 1 mg

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