Revised: 01–October–2004

Amplex® Red Catalase Assay Kit (A22180)

Quick Facts

Storage upon receipt:
- –20°C
- Desiccate
- Protect from light

Abs/Em of reaction product: 571/585 nm

Introduction

The Amplex® Red Catalase Assay Kit (A22180) provides an ultrasensitive yet simple assay for measuring catalase activity. Catalase is a heme-containing redox protein found in nearly all animal and plant cells as well as in aerobic microorganisms. In eukaryotic cells it is concentrated in the peroxisomes. Catalase is an important enzyme because H_2O_2 is a powerful oxidizing agent that is potentially damaging to cells. By preventing excessive H_2O_2 buildup, catalase allows important cellular processes which produce H_2O_2 as a by-product to take place safely.

In the assay, catalase first reacts with H_2O_2 to produce water and oxygen (O_2).[1] Next the Amplex Red reagent reacts with a 1:1 stoichometry with any unreacted H_2O_2 in the presence of horseradish peroxidase (HRP) to produce the highly fluorescent oxidation product, resorufin.[2,3] Therefore as catalase activity increases, the signal from resorufin decreases. The results are typically plotted by subtracting the observed fluorescence from that of a no-catalase control (Figure 1). Using the kit, one can detect catalase in a purified system at levels as low as 50 mU/mL.

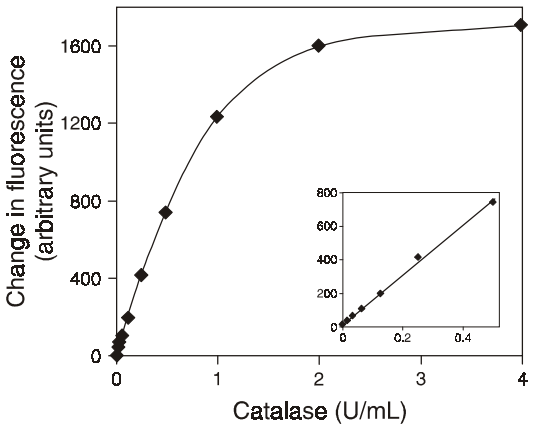

Figure 1. *Detection of catalase using the Amplex Red reagent–based assay. Initially each reaction contained the indicated amounts of catalase and 20 µM H_2O_2 in 1X Reaction Buffer and was incubated for 30 minutes. The final reaction containing 50 µM Amplex Red reagent and 0.2 U/mL HRP and was incubated at 37°C. After 30 minutes, fluorescence was measured in a fluorescence microplate reader using excitation at 530 ± 12.5 nm and fluorescence detection at 590 ± 17.5 nm. Change in fluorescence is reported as the observed fluorescence intensity subtracted from that of a no-catalase control.*

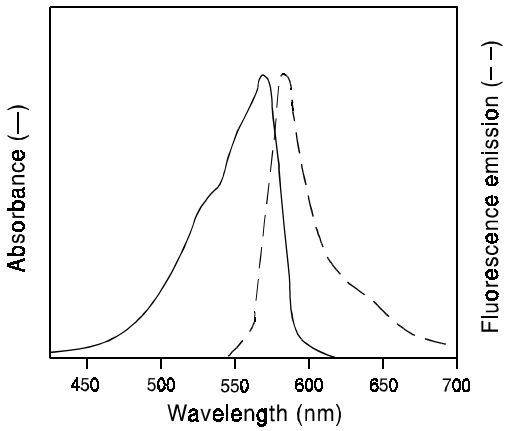

Figure 2. *Normalized absorption and fluorescence emission spectra.*

Resorufin has absorption and fluorescence emission maxima of approximately 571 nm and 585 nm, respectively (Figure 2). Because the absorbance is strong, the assay can be performed either fluorometrically or spectrophotometrically.

Materials

Kit Contents

- **Amplex Red reagent** (MW = 257, Component A) two vials, each containing 0.26 mg
- **Dimethylsulfoxide (DMSO), anhydrous** (Component B), 500 µL
- **Horseradish peroxidase** (Component C), 20 U, where 1 unit is defined as the amount of enzyme that will form 1.0 mg purpurogallin from pyrogallol in 20 seconds at pH 6.0 at 20°C
- **Hydrogen peroxide (H_2O_2)** (MW = 34, Component D), 500 µL of a stabilized ~3% solution; the actual concentration is indicated on the component label
- **5X Reaction Buffer** (Component E), 20 mL of 0.5 M Tris-HCl, pH 7.5)
- **Catalase** (Component F), 100 U, where 1 unit is defined as the amount of enzyme that will decompose 1.0 µmole of H_2O_2 per minute at pH 7.0 at 25°C

Each kit provides sufficient reagents for approximately 400 assays using either a fluorescence or absorbance microplate reader and reaction volumes of 100 µL per assay.

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.

Experimental Protocol

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 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.

Stock Solution Preparation

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 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 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 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 1000 U/mL solution of catalase by dissolving the contents of the vial of catalase (Component F) in 100 µL of dH_2O. After use, the remaining solution should be divided into small aliquots and stored frozen at -20°C.

Catalase Assay

The following protocol provides a guideline for using the Amplex Red Catalase Assay Kit to measure catalase activity. The volumes recommended here are sufficient for ~100 assays, each containing a volume of 100 µL.

2.1 Prepare a catalase standard curve: Dilute an appropriate amount of the 1000 U/mL catalase solution (prepared in step 1.5) into 1X Reaction Buffer to produce catalase concentrations of 0 to 4.0 U/mL. Use 1X Reaction Buffer without catalase as a negative control (Table 1). A volume of 25 µL will be used for each reaction. Please note that the catalase concentrations will be fourfold lower in the final reaction volume.

2.2 Dilute the catalase-containing samples in 1X Reaction Buffer. A volume of 25 µL will be used for each reaction. Please note that the samples' catalase concentrations will be fourfold lower in the final reaction volume.

2.3 Pipet 25 µL of the diluted experimental samples, standard curve samples and controls into separate wells of a 96-well microplate.

2.4 Prepare a 40 µM H_2O_2 solution by adding 10 µL of the 20 mM H_2O_2 solution (prepared in step 1.4) to 4.99 mL 1X Reaction Buffer

2.5 Pipet 25 µL of the 40 µM H_2O_2 solution to each microplate well containing the samples and controls.

2.6 Incubate the reaction for 30 minutes at room temperature.

2.7 Prepare a working solution of 100 µM Amplex Red reagent containing 0.4 U/mL HRP by adding 50 µL of the Amplex Red reagent stock solution (prepared in step 1.1) and 20 µL of the HRP stock solution (prepared in step 1.3) to 4.93 mL 1X Reaction Buffer. This 5 mL volume is sufficient for ~100 assays. Note that the final concentrations for the Amplex Red reagent and the HRP will be twofold lower in the final reaction volume.

Table 1. *Sample protocol for catalase standard curve.*

Volume of catalase solution *	Volume of 1X Reaction Buffer	Final catalase concentration †
0 µL	25 µL	0 mU/mL
6.25 µL of 1 U/mL	18.75 µL	62.5 mU/mL
12.5 µL of 1 U/mL	12.5 µL	125 mU/mL
2.5 µL of 10 U/mL	22.5 µL	250 mU/mL
5 µL of 10 U/mL	20 µL	500 mU/mL
10 µL of 10 U/mL	15 µL	1000 mU/mL

* Dilutions of the 1000 U/mL catalase solution should be made in the 1X Reaction Buffer. † The catalase solution is diluted fourfold in the final reaction volume.

2.8 Begin the second phase of the reaction by adding 50 µL of the Amplex Red/HRP working solution to each microplate well containing the samples and controls.

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

2.10 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 2).

2.11 Report the change in fluorescence or absorbance by subtracting the sample value from that of the no-catalase control (see Figure 1).

References

1. Anal Biochem 245, 55 (1997); **2.** Anal Biochem 253, 162 (1997); **3.** J Immunol Methods 202, 133 (1997).

Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
A22180	Amplex® Red Catalase Assay Kit *400 assays*	1 kit
A12222	Amplex® Red reagent (10-acetyl-3,7-dihydroxyphenoxazine)	5 mg
A22177	Amplex® Red reagent *packaged for high-throughput screening*	10 x 10 mg
A36006	Amplex® UltraRed reagent	5 x 1 mg

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