

**24,25(OH)₂D₃ and Regulation of
Catalase Activity in LNCaP Prostate
Cancer Cells: A Study of Long-term
Effects**

Project Work in Biomedicine, Advanced Level, 15 ECTS

(2007-08-20 – 2008-01-13)

ABSTRACT

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Department: School of Life Sciences, University of Skövde

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The vitamin D metabolite 1,25(OH)₂D₃ has long been known to inhibit growth of prostate cancer cells and this has been attributed to a VDR-mediated pathway controlling target gene expression, resulting in cell cycle arrest, apoptosis and differentiation. New research has shown that another vitamin D metabolite, 24,25(OH)₂D₃, inhibits proliferation of prostate cancer cells as well, more specifically, cells of the line LNCaP. It is not clear exactly how 24,25(OH)₂D₃ exerts this cancer growth inhibition but it has been shown that it is to some extent regulated via G protein coupled signalling pathways. Catalase is a haem-containing redox enzyme found in the majority of animal cells, plant cells and aerobic microorganisms. This enzyme is very important because it prevents excessive accumulation of the strongly oxidizing agent H₂O₂ which otherwise can do damage to the cells. Because of this preventive effect of catalase, important cellular processes which generate H₂O₂ as by-product can proceed safely. Biochemical analysis of catalase has shown that it binds endogenously to 24,25(OH)₂D₃. The fact that 24,25(OH)₂D₃ has anti-proliferative effects on prostate cancer cells combined with the fact that it binds to catalase generates the hypothesis that this binding interferes with the essential task of catalase to keep the cell free from accumulation of destructive H₂O₂, and by means of this interference induces apoptosis. Finding out about the cancer growth inhibiting mechanism behind each vitamin D metabolite is important and may be a lead in the search for a new, better treatment of prostate cancer. This is a follow-up to an earlier study, and the specific aim of this project was to find out if and in what way 24,25(OH)₂D₃ affects the enzymatic activity of catalase in LNCaP cells during long-term treatment (up to 48 hours). In this experiment LNCaP cells were incubated for 48 hours together with 24,25(OH)₂D₃ of the concentration 10⁻⁸ M, then a catalase assay was performed on the cells including fluorescence-mediated measuring of catalase activity in both treated and untreated cells. The analysis of the result values showed that despite of the rather high dose used, 24,25(OH)₂D₃ has no statistically significant effect on catalase activity in cells of the line LNCaP, regardless of time.

Table of Contents

1 Introduction	1
1.1 Prostate cancer.....	1
1.2 Vitamin D metabolites and prostate cancer.....	1
1.3 The enzyme catalase.....	2
1.4 Hypothesis.....	2
2 Aim of this Project Work.....	3
3 Materials and methods.....	3
3.1 Cell culturing.....	3
3.2 Treatment with 24,25(OH) ₂ D ₃	3
3.3 Catalase Assay, fluorescence measuring and computer analysis.....	4
3.3.1 <i>The Amplex Red Catalase Assay Kit, FluoStar Galaxy and GraphPad Prism 4</i>	<i>4</i>
3.3.2 <i>Assay, measuring and analysis.....</i>	<i>4</i>
4 Results and discussion.....	5
4.1 Long-term effects of 24,25(OH) ₂ D ₃ on catalase activity	5
4.2 Conclusion.....	6
5 Acknowledgements.....	6
6 References	6
6.1 Appendix	7

1 Introduction

1.1 Prostate cancer

The prostate is a gland located beneath the urinary bladder in men, surrounding the upper part of the urethra. In this gland a liquid is produced which nourishes the sperm and strengthens them. Both growth and function of the gland are regulated by the male hormone testosterone (Nystrand, 2005).

Cancer of the prostate is the most common form of male cancer. Each year, around half a million cases are diagnosed, worldwide. In Sweden, prostate cancer now accounts for a little more than 35% of all male cancers. The disease is much more common in Western countries than in for instance Asia, and in the USA, black people run a much greater risk of developing the disease than white people. Increasing age is another risk factor for this type of cancer (Nystrand, 2005).

It has been suggested that differences in vitamin D levels account for mentioned observations. Firstly, it is a fact that Japanese men consume larger amounts of fatty fish, the main dietary source of vitamin D, than do Western men (Zhao & Feldman, 2001). Secondly, light skin compared to dark contains less melanin, a compound in the skin which inhibits synthesis of vitamin D. Third, as men age their serum vitamin D levels decrease as the efficiency of vitamin D synthesis decreases with age (Holick, 2005). These suggestions are also supported by research showing that vitamin D has anti-proliferative effects on prostate cancer cells (discussed further below).

The symptoms of prostate cancer mainly consist of various urination difficulties and usually do not show until the tumor has spread outside the prostate capsule. The main medical treatments of this disease are prostatectomy, radiation therapy and testosterone ablation. Even though the prognosis of prostate cancer often is good, it is still the form of cancer linked to the highest death rates - in 2002, 2 352 Swedish men died with the disease – and the side-effects of the treatments are rather severe, the most common being impotence, incontinence and hot flushes (Nystrand, 2005). Because of this, more effective treatments with less side-effects are required.

1.2 Vitamin D metabolites and prostate cancer

The active form of Vitamin D is called $1,25(\text{OH})_2\text{D}_3$. It functions like a hormone in the body and is, together with the parathyroid hormone, a major regulator of mineral homeostasis and bone metabolism. $1,25(\text{OH})_2\text{D}_3$ aids intestinal calcium absorption and is important for prevention of diseases such as rickets and osteomalacia (Zhao & Feldman, 2001).

The metabolic chain forming $1,25(\text{OH})_2\text{D}_3$ begins with photolysis of 7-dehydrocholesterol by UV light which produces previtamin D_3 (Holick *et al.*, 1987). Thereafter, previtamin D_3 is hydroxylated at the 25-position in the liver, forming $25(\text{OH})\text{D}_3$ (Masumoto *et al.*, 1988), followed by 1-hydroxylation of $25(\text{OH})\text{D}_3$ in the kidneys (Lawson *et al.*, 1971). Alternatively a hydroxyl group is added to the side chain of $25(\text{OH})\text{D}_3$ or $1,25(\text{OH})_2\text{D}_3$, forming $24,25(\text{OH})_2\text{D}_3$ or $1,24,25(\text{OH})_2\text{D}_3$, respectively. Formation of $24,25(\text{OH})_2\text{D}_3$ and $1,24,25(\text{OH})_2\text{D}_3$ is believed to be the first inactivation step of the vitamin D metabolites as these products have lower biological activity than does $1,25(\text{OH})_2\text{D}_3$ (Akeno *et al.*, 1997).

The cellular receptor for $1,25(\text{OH})_2\text{D}_3$ is a nuclear receptor called the Vitamin D Receptor (VDR). The genes regulated upon binding with the VDR include genes important for calcium metabolism such as osteocalcin, osteopontin, 24-hydroxylase and calbindin (Haussler *et al.*, 1998) but also genes involved in cellular proliferation and differentiation such as c-myc, c-fos, p21, p27 and Hox A10 (Freedman, 1999).

Expressing VDR, the prostate, especially the tumorous prostate (Krill *et al.*, 2001), is a target organ for vitamin D and $1,25(\text{OH})_2\text{D}_3$ has long been known to inhibit growth of prostate cancer cells. This has been ascribed to a VDR-mediated pathway controlling target gene expression, resulting in cell cycle arrest, apoptosis and differentiation (Lou *et al.*, 2004). However, new research has shown that also $24,25(\text{OH})_2\text{D}_3$ inhibits proliferation of prostate cancer cells, more specifically, cells of a line called LNCaP (Hagberg, 2006; Sahlberg, 2006). These are cancer cells from the left supraclavicular lymphnode metastasis of a 50-year-old man with prostate carcinoma in 1977 (Horoszewicz, 1981) and they are commonly used in cancer research and drug investigation on which to test the effects of different agents (Winkler *et al.*, 2005). It is not clear exactly how $24,25(\text{OH})_2\text{D}_3$ exerts this cancer growth inhibition but it has been shown that it is to some extent regulated via G protein coupled signalling pathways (Björsson, 2006).

1.3 The enzyme catalase

Catalase is an enzyme, a haem-containing redox protein, which is found in the majority of animal cells, plant cells and aerobic microorganisms. It is concentrated mainly to the peroxisomes in eucaryotic cells. This enzyme is very important because it prevents excessive accumulation of the strongly oxidizing agent hydrogen peroxide (H_2O_2) which otherwise can do damage to the cells. Because of this preventive effect of catalase, important cellular processes which generate H_2O_2 as by-product can proceed safely (Zámocký & Koller, 1999).

Biochemical analysis of catalase has shown that it binds endogenously to three of the above presented vitamin D metabolites; $24,25(\text{OH})_2\text{D}_3$, $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$. The enzyme binds similarly effective to $24,25(\text{OH})_2\text{D}_3$ and $25(\text{OH})_2\text{D}_3$, while the binding capacity to $1,25(\text{OH})_2\text{D}_3$ is lower (Larsson *et al.*, 2006).

1.4 Hypothesis

The fact that vitamin D has anti-proliferative effects on prostate cancer cells combined with the fact that mentioned vitamin D metabolites, particularly $24,25(\text{OH})_2\text{D}_3$, bind to catalase generates a hypothesis. That is that this binding interferes with the essential task of catalase to keep the cell free from accumulation of destructive H_2O_2 , and by means of this interference induces apoptosis.

Finding out about the cancer growth inhibiting mechanism behind each vitamin D metabolite is important and may be a lead in the search for a new, better treatment of prostate cancer. This is a follow-up to an earlier study investigating the role, if any, of catalase in the mechanism behind the action of $24,25(\text{OH})_2\text{D}_3$ (Stahel, 2007).

2 Aim of this Project Work

The specific aim of this project was to study if and in what way $24,25(\text{OH})_2\text{D}_3$ affects the enzymatic activity of catalase in LNCaP cells during long-term treatment, that is, up to 48 hours.

3 Materials and methods

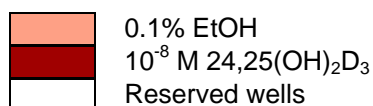
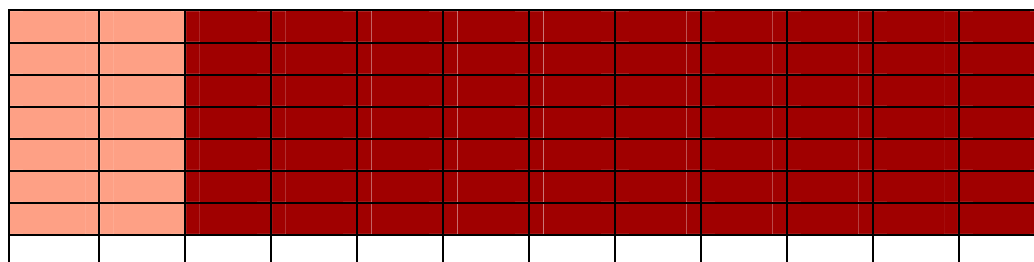
3.1 Cell culturing

Human prostate cancer cells from the cell line LNCaP clone FGC (ECACC, Salisbury, UK) were used for this experiment. They were grown in a single layer in cell culturing medium (CCM): RPMI 1640 medium, supplemented with 2 mM Glutamine, 10 mM HEPES, 1 mM Na-Pyruvate, 10% Fetal Bovine Serum and 100 U/ml Penicillin-Streptomycin. The culture was kept in 37°C in a humidified atmosphere with 5% CO_2 .

3.2 Treatment with $24,25(\text{OH})_2\text{D}_3$

84 wells of a 96 well microplate (Costar 96) were each filled with $198\ \mu\text{l}$ CCM containing 2 000 cells. The plate was then incubated in 37°C for 48 hours in order for the cells to form an attached layer in each well. To 70 of the 84 cell containing wells was then added CCM and $24,25(\text{OH})_2\text{D}_3$ yielding a final volume of $200\ \mu\text{l}$ in each well and a final $24,25(\text{OH})_2\text{D}_3$ concentration of $10^{-8}\ \text{M}$, according to Table 1. Finally, as controls, CCM and 99.5% EtOH was added to the last 14 cell containing wells giving a final volume of $200\ \mu\text{l}$ in each well and final pure EtOH concentration of 0.1%, again see Table 1. The microplate was then incubated in 37°C for 30 minutes.

Table 1: Treatment of the cells with $24,25(\text{OH})_2\text{D}_3$ using EtOH 0.1% as control. Final microplate well concentrations of $24,25(\text{OH})_2\text{D}_3$ and EtOH, respectively.



3.3 Catalase assay, fluorescence measuring and computer analysis

3.3.1 The Amplex Red Catalase Assay Kit, FluoStar Galaxy and GraphPad Prism 4

The Amplex Red Catalase Assay Kit (A22180) is a very sensitive, fluorescence-based assay used for measuring of catalase activity (Molecular Probes, 2004, Appendix).

In the first step of the assay, catalase reacts with H₂O₂, producing water and oxygen (O₂) (Mueller *et al.*, 1997). In the second step, an agent called the Amplex Red Reagent (ARR) reacts with any unreacted H₂O₂, in the presence of horseradish peroxidase (HRP), and this generates the highly fluorescent oxidation product resorufin (Zhou *et al.*, 1997; Mohanty *et al.*, 1997). This means that the higher catalase activity in the test, the weaker the resorufin signal gets. Resorufin has a very strong absorption capacity and because of this either a fluorometer or a spectrophotometer can be used for the assay (Molecular Probes, 2004, Appendix).

In this study a spectrofluorometer, FluoStar Galaxy (BMG Lab Technologies, Germany) was used for the testing. The FluoStar measures fluorescence in Arbitrary Fluorescence Units, AFU.

The computer program GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego CA, USA) was used for the statistical analysis of the result figures.

3.3.2 Assay, measuring and analysis

After the incubation the microplate was emptied by placing Kleenex on top of it then turning it upside-down. Steps 2.1–2.10 of the Amplex Red Catalase Assay procedure was then carried out according to the Amplex Red Catalase Assay Kit protocol (Molecular Probes, 2004, Appendix). The 37°C incubation time chosen for step 2.9 was 30 minutes, and the excitation and emission detection levels chosen for the fluorescence measuring in step 2.10 were set to 530 nm and 590 nm, respectively.

The fluorescence was measured at 9 time points: 0, 0.5, 1, 2, 4, 6, 15, 24 and 48 hours. The first measuring was done right after placing of the microplate in the FluoStar and at this time point the built-in incubation had reached the set temperature of 37°C.

The result values from the FluoStar were statistically analyzed in GraphPad Prism where a graph was drawn on basis of the result figures. The figures used for the graph were mean values, that is, an average was calculated for the 70 replicates with 10⁻⁸ M 24,25(OH)₂D₃ as well as the 14 EtOH controls. The analysis made was a F test. The significance threshold was set to P<0.05.

4 Results and discussion

4.1 Long-term effects of 24,25(OH)₂D₃ on catalase activity

The analyses of the result values in GraphPad Prism showed that in spite of the rather high dose used, 24,25(OH)₂D₃ had no statistically significant effect on catalase activity in LNCaP cells. As can be seen in Figure 1, there is no statistically significant difference between the slopes of the two curves representing treated cells and controls.

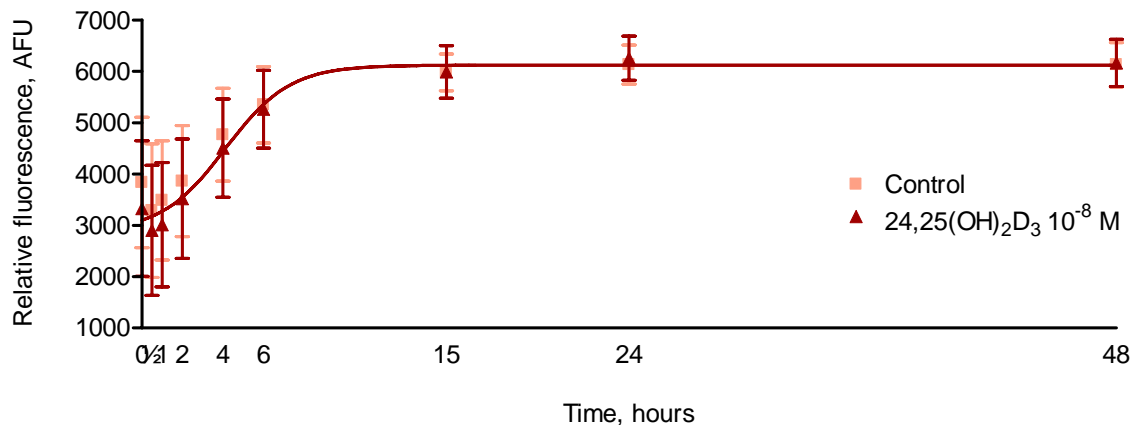


Figure 1: Time-dependent responses with and without 24,25(OH)₂D₃ treatment were compared with help of a F test. A fluorescence-based method was used where catalase activity was measured with Amplex Red Reagent.

The finding in this study that 24,25(OH)₂D₃ does not inhibit the H₂O₂ reducing property of catalase contradicts a study from 2006 in which Nemere *et al.* found that 24,25(OH)₂D₃ does inhibit catalase activity (Nemere *et al.*, 2006). This may be due to the fact that in Nemere's study chicken intestinal cells were used for the testing and not LNCaP cells as in this study. Also, in contrast to the cells in this study, the cells tested on in Nemere's study were not cancerous. In other words it may be that for unknown reasons, 24,25(OH)₂D₃ affects catalase activity in certain organs but not in others, or it may be that it affects non-cancerous tissue only. Alternatively, it could be that the hormone affects catalase in cells of certain species but not of others.

Perhaps the primary factors contributing to H₂O₂ decomposition besides catalase - increasing temperature, increasing pH, increasing contamination of transition metals and exposure to UV light (US Peroxide, 2002) - exist in other proportions in chicken intestine cells, and/or interact differently with catalase, than in human cells of the prostate.

Also there is the fact that vitamin D affects our biology in quite varying ways. It affects many different types of tissues and there are very diverging mechanisms behind its different effects (Norman, 2004). Because of this it is still possible that 24,25(OH)₂D₃ exerts its antiproliferative influence with help of catalase, even if not by effects on enzymatic activity. Possibly, catalase functions as a receptor for the hormone but affecting a different signalling pathway, today unknown. It may also be that that other proteins function as receptors for 24,25(OH)₂D₃.

4.2 Conclusion

The result from this study confirms the result from the above-mentioned earlier study investigating the possible role of catalase in the mechanism behind the action of 24,25(OH)₂D₃ (Stahel, 2007), namely that that 24,25(OH)₂D₃ does not affect the biology of prostate cancer cells via inhibition of catalase activity.

However, further investigations of if and in what way the hormone's binding to LNCaP cell catalase affects the enzyme or its activity are needed.

5 Acknowledgements

I would like to thank my supervisor, Dennis Larsson, for his as always kind, patient and trusting project guidance.

I would also like to thank Jonathan Holmén for giving me both great practical help and insights during my work with this study.

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6.1 Appendix

Molecular Probes (2004) *Amplex® Red Catalase Assay Kit Product Information.* Paisley: Invitrogen, Ltd.

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

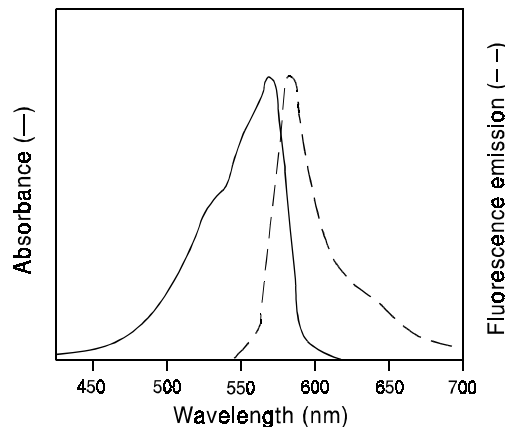


Figure 2. Normalized absorption and fluorescence emission spectra.

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 stoichiometry 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.

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.

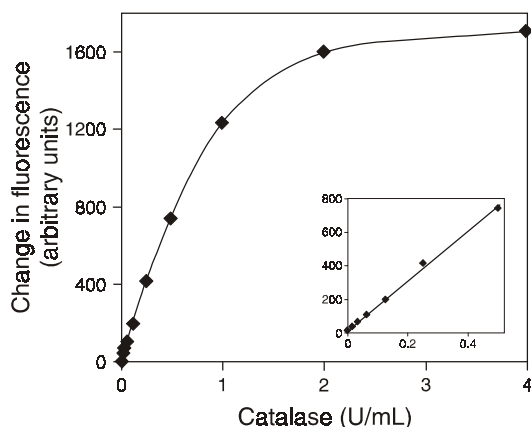


Figure 1. Detection of catalase using the Amplex Red reagent-based assay. Initially each reaction contained the indicated amounts of catalase and 20 μM H_2O_2 in 1X Reaction Buffer and was incubated for 30 minutes. The final reaction containing 50 μM Amplex Red reagent and 0.2 U/mL HRP and was incubated 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.

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 $\mu mole$ 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 $10\ \mu\text{M}$.

The absorption and fluorescence of resorufin are pH-dependent. Below the pK_a (~ 6.0), the absorption maximum shifts to $\sim 480\ \text{nm}$ 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 $\sim 3\%$ 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 $\sim 3\%$ 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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