ab83464
Catalase Activity Assay Kit (Colorimetric/Fluorometric)

For the rapid, sensitive and accurate measurement of catalase activity in various biological samples.

View kit datasheet: www.abcam.com/ab83464
(use www.abcam.cn/ab83464 for China, or www.abcam.co.jp/ab83464 for Japan)

This product is for research use only and is not intended for diagnostic use.
## Table of Contents

1. Overview 1
2. Protocol Summary 2
3. Precautions 3
4. Storage and Stability 3
5. Limitations 4
6. Materials Supplied 4
7. Materials Required, Not Supplied 5
8. Technical Hints 6
9. Reagent Preparation 7
10. Standard Preparation 8
11. Sample Preparation 10
12. Assay Procedure – Colorimetric Assay 12
14. Calculations 16
15. Typical data 18
16. Quick Assay Procedure 19
17. Troubleshooting 20
18. Interferences 22
19. FAQs 22
20. Notes 25
1. Overview

Catalase Activity Assay Kit (Colorimetric/Fluorometric) (ab83464) is a highly sensitive, simple and direct assay for measuring catalase activity in a variety of biological samples such as cell and tissue lysates or biological fluids. In this assay, the catalase present in the sample reacts with hydrogen peroxide (H₂O₂) to produce water and oxygen. The unconverted H₂O₂ reacts with probe to produce a product that can be measured colorimetrically at OD 570 nm or fluorometrically at Ex/Em = 535/587 nm. Therefore, the catalase activity present in the sample is reversely proportional to the signal obtained.

The kit can detect as little as 1 µU of catalase activity.

Catalase (EC 1.11.1.6) is a ubiquitous antioxidant enzyme that is present in nearly all living organisms. It catalyzes the decomposition of hydrogen peroxide (H₂O₂) to water and oxygen. Catalase is a tetramer of four polypeptide chains, and contains four porphyrin heme (iron) groups that allow the enzyme to react with hydrogen peroxide. The optimum pH for human catalase is approximately pH 7, with a fairly broad maximum as the rate of reaction does not change appreciably between pH= 6.8-7.5.
2. Protocol Summary

- Standard curve preparation & add Stop Solution
- Sample preparation
- Add H$_2$O$_2$ to samples & incubate 30 min at 25°C
- Add Stop solution to samples
- Add Development Mix & incubate at 25°C 10 min
- Measure absorbance (OD570 nm) or fluorescence (Ex/Em = 535/587 nm)
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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C 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 the Materials Supplied section.

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

⚠️ Note: Reconstituted components are stable for 2 months.
5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage temperature (before prep)</th>
<th>Storage temperature (after prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase Assay Buffer</td>
<td>25 mL</td>
<td>4°C</td>
<td>4°C</td>
</tr>
<tr>
<td>Catalase Positive Control</td>
<td>2 µL</td>
<td>4°C</td>
<td>4°C / -20 ºC</td>
</tr>
<tr>
<td>H₂O₂ (0.88 M)</td>
<td>25 µL</td>
<td>4°C</td>
<td>4°C</td>
</tr>
<tr>
<td>HRP (lyophilized)</td>
<td>1 vial</td>
<td>4°C</td>
<td>4°C</td>
</tr>
<tr>
<td>OxiRed probe (in DMSO)</td>
<td>200 µL</td>
<td>4°C</td>
<td>4°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 mL</td>
<td>4°C</td>
<td>4°C</td>
</tr>
</tbody>
</table>
7. 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 absorbance at OD 570 nm or fluorescence at Ex/Em = 535/587 nm
- MilliQ water or other type of double distilled water (ddH$_2$O)
- Cold PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom (colorimetric assay) / 96 well plate with clear flat bottom, preferably black (fluorometric assay)
- Dounce homogenizer (if using tissue)
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.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- 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 all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.
9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Catalase Assay Buffer:
Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.2 Catalase Positive Control:
Dilute the Catalase Positive Control in 500 µL of Catalase Assay Buffer and mix well by pipetting up and down. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C for 2 months or 2-3 days at 4°C. Keep on ice while in use.

9.3 H₂O₂ Standard:
Ready to use as supplied. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C protected from light. Keep on ice while in use.

9.4 HRP:
Reconstitute in 220 µL of Catalase Assay Buffer and mix well by pipetting up and down. Aliquot HRP so that you have enough volume to perform the desired number of assays. Store at 4°C. Once reconstituted, use within two months. Keep on ice while in use.

9.5 OxiRed Probe (in DMSO):
Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 min to thaw the DMSO solution before use.

△ Note: DMSO tends to be solid when stored at 4°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C.

Aliquot probe so that you have enough volume to perform the desired number of assays. Store at 4°C protected from light. Once the probe is thawed, use within two months.

9.6 Stop Solution:
Ready to use as supplied. Aliquot so that you have enough to perform the desired number of assays. Store at 4°C protected from light. Use within 2 months. Keep on ice while in use.
10. Standard Preparation

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

10.1 For colorimetric assay:

10.1.1 Prepare a 20 mM of H$_2$O$_2$ standard by diluting 5 µL of the provided H$_2$O$_2$ Standard (0.88 M solution) with 215 µL of ddH$_2$O.

10.1.2 Prepare a 1 mM H$_2$O$_2$ standard by diluting 50 µL of the 20 mM H$_2$O$_2$ Standard with 950 µL of ddH$_2$O.

10.1.3 Using 1 mM H$_2$O$_2$ standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>H$_2$O$_2$ Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End amount H$_2$O$_2$ in well (nmol/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>270</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>264</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>258</td>
<td>90</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>252</td>
<td>90</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>246</td>
<td>90</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>240</td>
<td>90</td>
<td>10</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (2 x 90 µL).

10.1.4 Add 10 µL of Stop Solution into each standard well.
10.2 For fluorometric assay:

*Note*: Detection sensitivity of fluorometric assay is 10-100 fold higher than colorimetric assay.

10.2.1 Prepare a 20 mM of H$_2$O$_2$ standard by diluting 5 µL of the provided H$_2$O$_2$ Standard (0.88 M solution) with 215 µL of ddH$_2$O.

10.2.2 Prepare a 1 mM H$_2$O$_2$ standard by diluting 50 µL of the 20 mM H$_2$O$_2$ Standard with 950 µL of ddH$_2$O.

10.2.3 Prepare a 0.1 mM H$_2$O$_2$ standard by diluting 100 µL of the 1 mM H$_2$O$_2$ Standard with 900 µL of ddH$_2$O.

10.2.4 Using 0.1 mM H$_2$O$_2$ standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>H$_2$O$_2$ Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End amount H$_2$O$_2$ in well (nmol/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>270</td>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>264</td>
<td>90</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>258</td>
<td>90</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>252</td>
<td>90</td>
<td>0.6</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>246</td>
<td>90</td>
<td>0.8</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>240</td>
<td>90</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (2 x 90 µL).

10.2.5 Add 10 µL of Stop Solution into each standard well.

*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 snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Reducing agents present in the sample will interfere with the assay. Keep DTT or β-mercaptoethanol below 5 mM.

11.1 Cell lysates:
11.1.1 Harvest the amount of cells (adherent/suspension) necessary for each assay (initial recommendation: 10^6 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend cells in 200 µL of ice-cold Assay Buffer.
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Centrifuge 15 minutes at 4°C at 10,000 x g in a cold microcentrifuge to remove any insoluble material.
11.1.6 Collect supernatant and transfer to a new tube.
11.1.7 Keep on ice.

11.2 Tissue lysates:
11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation: 100 mg).
11.2.2 Wash tissue in cold PBS.
11.2.3 Add 200 µL of ice-cold Assay Buffer to tissue.
11.2.4 Homogenize tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.
11.2.5 Centrifuge sample for 15 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.
11.2.6 Collect supernatant and transfer to a new tube.
11.2.7 Keep on ice.
11.3 **Plasma, Serum and Urine (and other biological fluids):**

Plasma, Serum and urine samples can be tested directly by adding samples to the microplate wells. However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the samples.

11.4 **Erythrocytes:**

11.4.1 Harvest the amount of cells necessary for each assay (initial recommendation: 200 μL).
11.4.2 Wash cells with cold PBS.
11.4.3 Resuspend cells in 200 μL of ice cold Assay Buffer.
11.4.4 Homogenize cells quickly by pipetting up and down a few times.
11.4.5 Centrifuge sample for 15 minutes at 4°C at 10,000 xg using a cold microcentrifuge to remove any insoluble material.
11.4.6 Collect supernatant and transfer to a new tube.
11.4.7 Keep on ice.

**Note:** We suggest using different volumes of sample to ensure readings are within the standard curve range.
12. Assay Procedure – Colorimetric Assay

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

△ Note: Reducing agents such as DTT or β-mercaptoethanol will interfere with the assay if present at concentrations > 5 µM.

12.1 Plate Loading:
- Standard wells = 100 µL standard dilutions [90 µL Standard + 10 µL Stop Solution].
- Positive Control = 1-5 µL Positive Control (adjust volume to 78 µL/well with Catalase Assay Buffer).
- Sample wells = 2-78 µL samples (adjust volume to 78 µL/well with Catalase Assay Buffer).
- Sample High Control (HC) wells = 2-78 µL samples (adjust volume to 78 µL/well with Catalase Assay Buffer).

12.2 HC Catalase inhibition:
12.2.1 Add 10 µL Stop Solution into each Sample HC wells. Do not add to any other wells.
12.2.2 Mix well and incubate at 25°C for 5 minutes to completely inhibit the catalase activity in the sample HC wells.

12.3 Catalase Reaction:
12.3.1 Add 12 µL of fresh 1 mM H₂O₂ solution (see Step 10.1.2) into each sample, positive control and sample HC wells.
12.3.2 Incubate reaction at 25°C for 30 minutes.
△ Note: Addition of extra H₂O₂ in the sample ensures that the readings of the HC and sample wells fit within the standard curve range in case they contain a lot of catalase.
12.3.3 Add 10 µL Stop Solution to each sample and positive control wells. Do not add Stop Solution to standard dilution or to Sample HC wells (it has already been added in step 12.2).
12.4 Developer Mix:

12.4.1 Prepare 50 µL of Developer Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix of the Developer mix to ensure consistency.

<table>
<thead>
<tr>
<th>Component</th>
<th>Developer Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase Assay Buffer</td>
<td>46</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>2</td>
</tr>
<tr>
<td>HRP Solution</td>
<td>2</td>
</tr>
</tbody>
</table>

12.4.2 Add 50 µL of Developer Mix into each standard, sample, sample HC and positive control wells.
12.4.3 Mix and incubate at 25°C for 10 min protected from light.

12.5 Measurement:

12.5.1 Measure output immediately at OD 570 nm on a microplate reader.

⚠️ Note: For low amounts of catalase, you can either increase the incubation time prior to adding the Stop Solution (Step 12.3.2) or use the fluorometric assay.
13. Assay Procedure – Fluorometric Assay

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

△ Note: Reducing agents such as DTT or β-mercaptoethanol will interfere with the assay if present at concentrations > 5 µM.

13.1 Plate Loading:
- Standard wells = 100 µL standard dilutions [90 µL Standard + 10 µL Stop Solution].
- Sample wells = 2-78 µL samples (adjust volume to 78 µL/well with Catalase Assay Buffer).
- Positive Control = 1-5 µL Positive Control (adjust volume to 78 µL/well with Catalase Assay Buffer).
- Sample High Control (HC) wells = 2-78 µL samples (adjust volume to 78 µL/well with Catalase Assay Buffer).

13.2 HC Catalase inhibition:
13.2.1 Add 10 µL Stop Solution into each Sample HC wells. Do not add to any other wells.
13.2.2 Mix well and incubate at 25ºC for 5 minutes to completely inhibit the catalase activity in the sample HC wells.

13.3 Catalase Reaction:
13.3.1 Prepare Catalase Reaction for each sample, positive control and sample HC wells by mixing 1.5 µL fresh 1 mM H₂O₂ solution (see Step 10.2.2) with 10.5 µL Catalase Assay Buffer. Prepare a master mix to ensure consistency.
13.3.2 Add 12 µL diluted H₂O₂ solution into each sample, positive control and sample HC wells.
13.3.3 Incubate reaction at 25ºC for 30 minutes.

△ Note: Addition of extra H₂O₂ in the sample ensures that the readings of the HC and sample wells fit within the standard curve range in case they contain a lot of catalase.
13.3.4 Add 10 µL Stop Solution to each sample and positive control wells. Do Stop Solution to standard dilution or to Sample HC wells (it has already been added in step 13.2).

13.4 Developer Mix:
13.4.1 Prepare 50 µL of Developer Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix of the Developer mix to ensure consistency.

<table>
<thead>
<tr>
<th>Component</th>
<th>Developer Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase Assay Buffer</td>
<td>47.7</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>0.3</td>
</tr>
<tr>
<td>HRP Solution</td>
<td>2</td>
</tr>
</tbody>
</table>

13.4.2 Add 50 µL of Developer Mix into each standard, sample, sample HC and positive control wells.
13.4.3 Mix and incubate at 25°C for 10 min protected from light.

13.5 Measurement:
13.5.1 Measure output immediately at Ex/Em = 535/587 nm on a microplate reader.

**Note:** For low amounts of catalase, increase the incubation time prior to adding the Stop Solution (Step 13.3.3).
14. Calculations

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

- Use only the linear rate for calculation.

14.1 Subtract the mean absorbance/fluorescence value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance (OD)/fluorescence (RFU).

14.2 Average the duplicate reading for each standard and sample.

14.3 Plot the corrected standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

14.4 Calculate $\Delta$OD/$\Delta$RFU signal in the sample as follows:

$$\Delta \text{OD} = A_{HC} - A_{\text{Sample}}$$
$$\Delta \text{RFU} = \text{RFU}_{HC} - \text{RFU}_{\text{Sample}}$$

Where “HC” is the reading of the sample High Control, “Sample” is the reading of the sample.

14.5 Apply the $\Delta$OD/$\Delta$RFU to $H_2O_2$ Standard Curve (colorimetric or fluorometric as per assay) to get B nmol of $H_2O_2$ decomposed by catalase during the 30 min reaction.

14.6 Catalase activity (nmol/min/mL or mU/mL) in the test samples is calculated as:

$$\text{Catalase Activity} = \left( \frac{B}{30 \times V} \right) \times D$$

Where:

B = amount of $H_2O_2$ in sample well calculated from standard curve (nmol).

30 = Catalase reaction time (minutes) – see Step 12.3.2 or 13.3.3.

V = preoriginal sample volume added into the reaction well (mL).

D = sample dilution factor if sample diluted further to fit within standard curve reading.
Unit definition:
1 Unit Catalase activity = amount of catalase that will decompose
1.0 µmol of H₂O₂ per minute at pH 4.5 at 25°C.
15. 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 H₂O₂ standard calibration curve using colorimetric reading.

Figure 2. High control and sample readings obtained using colorimetric reading.
16. 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 reagents (aliquot if necessary); get equipment ready.
- Prepare H$_2$O$_2$ standard dilution for your desired detection method: colorimetric [2 – 10 nmol/well] or fluorometric [0.2.-1 nmol/well]. Add 10 µL of Stop Solution to standard.
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (100 µL), samples (78 µL), positive control (78 µL) and sample High Control (HC) (78 µL).
- Sample HC only: add 10 µL of Stop Solution and incubate at 25ºC for 5 minutes.
- Add 12 µL H$_2$O$_2$ 1 mM solution (colorimetric) or 1.5 µL H$_2$O$_2$ 1 mM solution + 10.5 µL Assay Buffer (fluorometric) into each sample, positive control and sample HC well. Incubate at 25ºC for 30 min.
- Add 10 µL Stop Solution into each sample and positive control well.
- Prepare a master mix for Developer Mix:

<table>
<thead>
<tr>
<th>Component</th>
<th>Colorimetric Developer Mix (µL)</th>
<th>Fluorometric Developer Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay Buffer</td>
<td>46</td>
<td>47.7</td>
</tr>
<tr>
<td>OxiRed Probe</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>HRP Solution</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
- Add 50 µL Developer Mix into each well.
- Incubate plate at 25ºC for 10 minutes.
- Measure plate at OD 570 nm/Ex/Em= 535/587 nm on a microplate reader.
## 17. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay not working</strong></td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at assay temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Use of a different microplate</td>
<td>Colorimetric: clear plates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fluorometric: black wells/clear bottom plates</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Luminometric: white wells/clear bottom plates</td>
</tr>
<tr>
<td><strong>Sample with erratic readings</strong></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer, increase number of strokes</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple free/thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>Check protocol for interfering substances</td>
</tr>
<tr>
<td><strong>Lower/higher readings in samples and standards</strong></td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Allowing reagents to sit for extended times on ice</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td>Problem</td>
<td>Reason</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Standard readings do not follow a linear pattern</strong></td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes (&lt; 5 µL) and prepare a master mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions described in the protocol</td>
</tr>
<tr>
<td><strong>Unanticipated results</strong></td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/ below the linear range</td>
<td>Concentrate/ Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
18. Interferences

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:
- Reducing agents: DTT or β-mercaptoethanol present at > 5 µM.

19. FAQs

Q. The concentration of H₂O₂ added to wells via the catalase reaction is 1.5X higher than the top dose used for the standard curve. Therefore, it cannot be assumed that the fluorescence of the HC wells is an accurate value to calculate the activity from. Could you please explain the reason for this?
A. The reason to add extra H₂O₂ in the sample is to ensure that the readings of the HC and sample wells fit within the standard curve. For example, on figure 2 shown on the datasheet, the sample data starts with OD 1.5 (HC) while the sample OD is 0.25. The difference in OD values is therefore almost near the upper end of the standard curve. It is to help ensure the sample values remain in the range of the assay if they contain a lot of catalase.

Q. Will the kit work with bacterial samples?
A. The kit has been tested in human samples. However, it can be adapted to work with bacterial cells.
For Gram positive bacteria, lysozyme treatment might be required to rupture the cell wall.
For Gram negative bacteria, follow protocol for preparing cell lysate.
We recommend testing different dilutions of the sample to make sure the final readings are within the linear range of the standard curve.

Q. Will the kit work with food samples?
A. The kit can be adapted to work with food samples.
Solid samples should be homogenized in Assay Buffer and centrifuge to collect supernatant.
Liquid samples do not need any additional preparation step but we recommend a quick centrifugation step to ensure there is no floating debris or particulate material.
We recommend testing different dilutions of the sample to make sure the final readings are within the linear range of the standard curve.

**Q. What is the activity level of the positive control? How can we increase its value to be comparable with our samples?**

A. The positive control is only a benchmark sample. As long as the values are within the range of the standard curve is fine. The positive control is provided to validate that the assay components are working, not to be used for comparison with samples. The more positive control added to the wells, the higher the reading values, but you sure ensure they still fall within the range of the standard curve.

**Q. The RFU values are the same for increasing volumes of our sample. Why?**

A. The classic cue to saturation is that when you add more sample the value decreases, meaning the maximum has already been attained and there is either limitation of reagents or Vmax has been reached already.

When there is high amount of catalase in the sample, all the substrate is quickly converted into product and then substrate is no longer available, limiting the color development. When you dilute the sample, there is less catalase and hence the substrate is gradually converted to product showing a gradual increase over time. Sample volume needs to be optimized to make sure that just enough is used to get values in the linear range of the standard curve, not too high or not low.
Q. What is the difference between the Catalase Activity Assay Kit (ab83464) and the Peroxidase Activity Assay Kit (ab155895), since both quantify $\text{H}_2\text{O}_2$?

A. Catalase causes degradation of H2O2 while peroxidase uses H2O2 as substrate. When looking at catalase activity, OD/RFU will decrease whereas when looking at peroxidase activity, the OD/RFU will increase until enough substrate is available.

You can study the activity of catalase in presence of peroxidase using ab83464. However, if catalase is present in the sample while using ab155895 to detect peroxidase activity, catalase will cause a reduction in the signal and will result in underestimation of peroxidase activity.
20. Notes
Technical Support

Copyright © 2009-2017 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259

France
supportscientifique@abcam.com | 01.46.94.62.96

Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154

Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com

UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
c.ca.technical@abcam.com | 877-749-8807

US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823

China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500

Japan
technical@abcam.co.jp | +81-(0)3-6231-0940

Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450

New Zealand
nz.technical@abcam.com | +64-(0)9-909-7829