

### ab83464 Catalase Activity Assay Kit (Colorimetric/Fluorometric)

For the rapid, sensitive and accurate measurement of catalase activity in various biological samples. This product is for research use only and is not intended for diagnostic use.

**For overview, typical data and additional information please visit: [www.abcam.com/ab83464](http://www.abcam.com/ab83464)** (use [abcam.cn/ab83464](http://abcam.cn/ab83464) for China, or [abcam.co.jp/ab83464](http://abcam.co.jp/ab83464) for Japan)

#### Materials Supplied and Storage

**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.

**Δ Note:** Reconstituted components are stable for 2 months.

| Item                                   | Quantity | Storage temperature (before prep) | Storage temperature (after prep) |
|--|----------|-----------------------------------|----------------------------------|
| Catalase Assay Buffer                  | 25 mL    | 4°C                               | 4°C                              |
| Catalase Positive Control              | 2 µL     | 4°C                               | 4°C / -20 °C                     |
| H <sub>2</sub> O <sub>2</sub> (0.88 M) | 25 µL    | 4°C                               | 4°C                              |
| HRP (lyophilized)                      | 1 vial   | 4°C                               | 4°C                              |
| OxiRed probe (in DMSO)                 | 200 µL   | 4°C                               | 4°C                              |
| Stop Solution                          | 1 mL     | 4°C                               | 4°C                              |

#### Materials Required, Not Supplied

- 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<sub>2</sub>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)

#### 1. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

- 1.1 Catalase Assay Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.
- 1.2 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.
- 1.3 H<sub>2</sub>O<sub>2</sub> 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.
- 1.4 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.
- 1.5 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.

**Δ 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.

- 1.6 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.

#### 2. Standard Preparation

Always prepare a fresh set of standards. Discard working standard dilutions after use.

##### 2.1 For colorimetric assay:

- 2.1.1 Prepare a 20 mM of H<sub>2</sub>O<sub>2</sub> standard by diluting 5 µL of the provided H<sub>2</sub>O<sub>2</sub> Standard (0.88 M solution) with 215 µL of ddH<sub>2</sub>O.
- 2.1.2 Prepare a 1 mM H<sub>2</sub>O<sub>2</sub> standard by diluting 50 µL of the 20 mM H<sub>2</sub>O<sub>2</sub> Standard with 950 µL of ddH<sub>2</sub>O.
- 2.1.3 Using 1 mM H<sub>2</sub>O<sub>2</sub> standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

| Standard# | H <sub>2</sub> O <sub>2</sub> Standard (µL) | Assay Buffer (µL) | Final volume standard in well (µL) | End amount H <sub>2</sub> O <sub>2</sub> in well (nmol/well) |
|-----------|---|-------------------|------------------------------------|--|
| 1         | 0   | 270               | 90                                 | 0  |
| 2         | 6   | 264               | 90                                 | 2  |
| 3         | 12  | 258               | 90                                 | 4  |
| 4         | 18  | 252               | 90                                 | 6  |
| 5         | 24  | 246               | 90                                 | 8  |
| 6         | 30  | 240               | 90                                 | 10   |

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

- 2.1.4 Add 10 µL of Stop Solution into each standard well.

##### 2.2 For fluorometric assay:

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

- 2.2.1 Prepare a 20 mM of H<sub>2</sub>O<sub>2</sub> standard by diluting 5 µL of the provided H<sub>2</sub>O<sub>2</sub> Standard (0.88 M solution) with 215 µL of ddH<sub>2</sub>O.
- 2.2.2 Prepare a 1 mM H<sub>2</sub>O<sub>2</sub> standard by diluting 50 µL of the 20 mM H<sub>2</sub>O<sub>2</sub> Standard with 950 µL of ddH<sub>2</sub>O.
- 2.2.3 Prepare a 0.1 mM H<sub>2</sub>O<sub>2</sub> standard by diluting 100 µL of the 1 mM H<sub>2</sub>O<sub>2</sub> Standard with 900 µL of ddH<sub>2</sub>O.
- 2.2.4 Using 0.1 mM H<sub>2</sub>O<sub>2</sub> standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

| Standard# | H <sub>2</sub> O <sub>2</sub> Standard (µL) | Assay Buffer (µL) | Final volume standard in well (µL) | End amount H <sub>2</sub> O <sub>2</sub> in well (nmol/well) |
|-----------|---|-------------------|------------------------------------|--|
| 1         | 0   | 270               | 90                                 | 0  |
| 2         | 6   | 264               | 90                                 | 0.2  |
| 3         | 12  | 258               | 90                                 | 0.4  |
| 4         | 18  | 252               | 90                                 | 0.6  |
| 5         | 24  | 246               | 90                                 | 0.8  |
| 6         | 30  | 240               | 90                                 | 1.0  |

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

- 2.2.5 Add 10 µL of Stop Solution into each standard well.

**Δ 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.

#### 3. Sample Preparation

- 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  $\beta$ -mercaptoethanol below 5 mM.

### 3.1 Cell lysates:

- 3.1.1 Harvest the amount of cells (adherent/suspension) necessary for each assay (initial recommendation:  $10^6$  cells).
- 3.1.2 Wash cells with cold PBS.
- 3.1.3 Resuspend cells in 200  $\mu$ L of ice-cold Assay Buffer.
- 3.1.4 Homogenize cells quickly by pipetting up and down a few times.
- 3.1.5 Centrifuge 15 minutes at 4°C at 10,000 xg in a cold microcentrifuge to remove any insoluble material.
- 3.1.6 Collect supernatant and transfer to a new tube. Keep on ice.

### 3.2 Tissue lysates:

- 3.2.1 Harvest the amount of tissue necessary for assay (initial recommendation: 100 mg).
- 3.2.2 Wash tissue in cold PBS.
- 3.2.3 Add 200  $\mu$ L of ice-cold Assay Buffer to tissue.
- 3.2.4 Homogenize tissue with a Dounce homogenizer or pestle on ice, with 10 – 15 passes.
- 3.2.5 Centrifuge sample for 15 minutes at 4°C at 10,000 xg using a cold microcentrifuge to remove any insoluble material.
- 3.2.6 Collect supernatant and transfer to a new tube. Keep on ice.

### 3.3 Plasma, Serum and Urine (and other biological fluids):

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

### 3.4 Erythrocytes:

- 3.4.1 Harvest the amount of cells necessary for assay (initial recommendation: 200  $\mu$ L).
- 3.4.2 Wash cells with cold PBS.
- 3.4.3 Resuspend cells in 200  $\mu$ L of ice cold Assay Buffer.
- 3.4.4 Homogenize cells quickly by pipetting up and down a few times.
- 3.4.5 Centrifuge sample for 15 minutes at 4°C at 10,000 xg using a cold microcentrifuge to remove any insoluble material.
- 3.4.6 Collect supernatant and transfer to a new tube. Keep on ice.

**Δ Note:** We suggest using different volumes of sample to ensure readings are within the standard curve range.

## 4. 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.

**Δ Note:** Reducing agents such as DTT or  $\beta$ -mercaptoethanol will interfere with the assay if present at concentrations > 5  $\mu$ M.

### 4.1 Plate Loading:

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

### 4.2 HC Catalase inhibition:

- 4.2.1 Add 10  $\mu$ L Stop Solution into each Sample HC wells. Do not add to any other wells.
- 4.2.2 Mix well and incubate at 25°C for 5 minutes to completely inhibit the catalase activity in the sample HC wells.

### 4.3 Catalase Reaction:

- 4.3.1 Add 12  $\mu$ L of fresh 1 mM  $H_2O_2$  solution (see Step 2.1.2) into each sample, positive control and sample HC wells.
- 4.3.2 Incubate reaction at 25°C for 30 minutes.  
**Δ Note:** Addition of extra  $H_2O_2$  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.
- 4.3.3 Add 10  $\mu$ L Stop Solution to each sample and positive control wells. Do not add Stop Solution to standard dilution or to Sample HC wells (it has been added in step 4.2).

### 4.4 Developer Mix:

- 4.4.1 Prepare 50  $\mu$ L 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.

| Component             | Developer Mix ( $\mu$ L) |
|-----------------------|--------------------------|
| Catalase Assay Buffer | 46                       |
| OxiRed Probe          | 2                        |
| HRP Solution          | 2                        |

- 4.4.2 Add 50  $\mu$ L of Developer Mix into each standard, sample, sample HC and positive control wells.
- 4.4.3 Mix and incubate at 25°C for 10 min protected from light.

### 4.5 Measurement:

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

**Δ Note:** For low amounts of catalase, you can either increase the incubation time prior to adding the Stop Solution (Step 4.3.2) or use the fluorometric assay.

## 5. 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.

**Δ Note:** Reducing agents such as DTT or  $\beta$ -mercaptoethanol will interfere with the assay if present at concentrations > 5  $\mu$ M.

### 5.1 Plate Loading:

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

### 5.2 HC Catalase inhibition:

- 5.2.1 Add 10  $\mu$ L Stop Solution into each Sample HC wells. Do not add to any other wells.
- 5.2.2 Mix well and incubate at 25°C for 5 minutes to completely inhibit the catalase activity in the sample HC wells.

### 5.3 Catalase Reaction:

- 5.3.1 Prepare Catalase Reaction for each sample, positive control and sample HC wells by mixing 1.5  $\mu$ L fresh 1 mM  $H_2O_2$  solution (see Step 2.2.2) with 10.5  $\mu$ L Catalase Assay Buffer. Prepare a master mix to ensure consistency.
- 5.3.2 Add 12  $\mu$ L diluted  $H_2O_2$  solution into sample, positive control and sample HC wells.
- 5.3.3 Incubate reaction at 25°C for 30 minutes.

**Δ Note:** Addition of extra H<sub>2</sub>O<sub>2</sub> in the sample ensures that the readings of the HC and sample wells fit within the standard curve range if they contain a lot of catalase.

- 5.3.4 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 5.2).

#### 5.4 Developer Mix:

- 5.4.1 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.

| Component             | Developer Mix (μL) |
|-----------------------|--------------------|
| Catalase Assay Buffer | 47.7               |
| OxiRed Probe          | 0.3                |
| HRP Solution          | 2                  |

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

#### 5.5 Measurement:

- 5.5.1 Measure output immediately at Ex/Em = 535/587 nm on a microplate reader.

**Δ Note:** For low amounts of catalase, increase the incubation time prior to adding the Stop Solution (Step 5.3.3).

### 6. 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.

- 6.1 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).
- 6.2 Average the duplicate reading for each standard and sample.
- 6.3 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).
- 6.4 Calculate ΔOD/ΔRFU signal in the sample as follows:  

$$\Delta OD = A_{HC} - A_{Sample}$$

$$\Delta RFU = RFU_{HC} - RFU_{Sample}$$
 Where "HC" is the reading of the sample High Control, "Sample" is the reading of the sample.
- 6.5 Apply the ΔOD/ΔRFU to H<sub>2</sub>O<sub>2</sub> Standard Curve (colorimetric or fluorometric as per assay) to get B nmol of H<sub>2</sub>O<sub>2</sub> decomposed by catalase during the 30 min reaction.
- 6.6 Catalase activity (nmol/min/mL or mU/mL) in the test samples is calculated as:

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

Where:

B = amount of H<sub>2</sub>O<sub>2</sub> in sample well calculated from standard curve (nmol).

30 = Catalase reaction time (minutes) – see Step 4.3.2 or 5.3.3.

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

D = sample dilution factor if sample diluted further to fit within standard curve reading.

1 Unit Catalase activity = amount of catalase that will decompose 1.0 μmol of H<sub>2</sub>O<sub>2</sub> per minute at pH 4.5 at 25°C.

**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.

### FAQs

**Q. The concentration of H<sub>2</sub>O<sub>2</sub> 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?** The reason to add extra H<sub>2</sub>O<sub>2</sub> 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?** 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?** 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?** 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?** 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 V<sub>max</sub> 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 H<sub>2</sub>O<sub>2</sub>?** Catalase causes degradation of H<sub>2</sub>O<sub>2</sub> while peroxidase uses H<sub>2</sub>O<sub>2</sub> 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.

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