ab83464 – Catalase Activity Assay Kit (Colorimetric/Fluorometric)

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

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

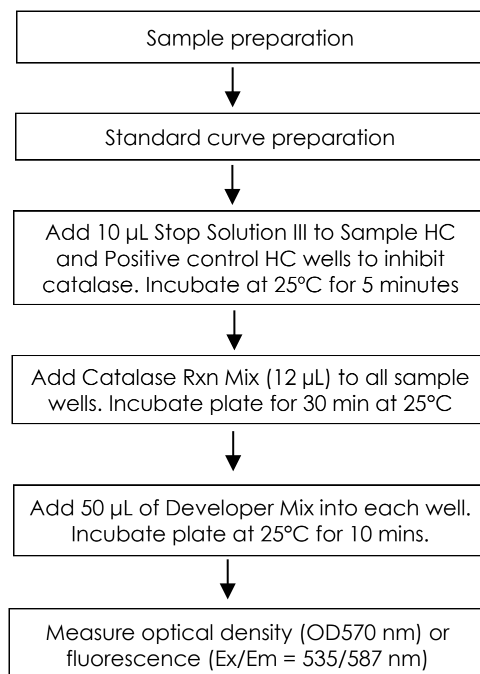
Background:

Catalase Assay Kit (ab83464) provides a sensitive, direct and automation ready procedure for quantifying catalase activity in tissue lysates, cell lysates, plasma, and serum.

In the assay, catalase first reacts with H_2O_2 to produce water and oxygen, the unconverted H_2O_2 reacts with OxiRed™ probe to produce a product, which can be measured at 570 nm (colorimetric method) or at Ex/Em = 535/587 nm (fluorometric method). Catalase activity is reversely proportional to the signal. The kit can detect 1 µU or less of catalase activity in sample

Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.



QUICK ASSAY PROCEDURE

- Solubilize Developer Solution V, thaw Assay Buffer 8, Catalase Positive Control, Hydrogen Peroxide Solution III, OxiRed™ probe and Stop Solution III
- Prepare samples in duplicate
- Prepare appropriate standard curve (colorimetric or fluorometric).
- HC Catalase inhibition: Add 10 µL Stop Solution III into each Sample HC and Positive control HC wells. Incubate at 25°C for 5 minutes.
- Catalase reaction: Add 12 µL of catalase reaction mix into each sample, sample HC, positive control, and positive control well.
- Incubate reaction at 25°C for 30 minutes.
- Add 10 µL Stop Solution III to each sample and positive control wells.
- Add 50 µL of Developer Mix into each well.
- Incubate plate at 25°C for 10 mins.
- Measure plate at OD 570nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

Precautions & Limitations:

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.

- Modifications to the kit components or procedures may result in loss of performance.
- 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.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Assay Buffer 8	25 mL	-20 °C	4°C
Catalase Positive Control	20 µL	-20 °C	-20 °C
Hydrogen Peroxide Solution III	25 µL	-20 °C	4°C
Developer Solution V	1 vial	-20 °C	-20°C
OxiRed™ probe	0.2 mL	-20 °C	4°C
Stop Solution III	1 mL	-20 °C	20°C

PLEASE NOTE: Assay Buffer 8 was previously labeled as Assay Buffer VIII and Catalase Assay Buffer, and OxiRed™ probe as OxiRed Probe and OxiRed probe (in DMSO). Also, Stop Solution III as Stop Solution, and Developer Solution V as HRP (lyophilized), and Hydrogen Peroxide Solution III as H_2O_2 (0.88 M). The composition has not changed.

Materials Required, Not Supplied:

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance (OD) at 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- 96 well clear plate with clear flat bottom (colorimetric assay) / 96 well black plate with flat bottom (fluorometric assay)
- Orbital shaker
- Microcentrifuge
- Dounce homogenizer (if using cells or tissue)
- 1 x PBS pH 7.4 (ab285410 or similar)
- MilliQ water or other type of double distilled/deionized water (ddH_2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

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Assay Buffer 8: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Catalase Positive Control: Dilute 2 µL of Catalase Positive Control in 50 µL of Assay Buffer 8 and mix well by pipetting up and down before use. Undiluted stock must be stored at -20°C. Diluted positive control is stable 2-3 days at 4°C. Do not freeze thaw diluted positive control. Keep on ice while in use.

Hydrogen Peroxide Solution III: 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.

Developer Solution V**:** Reconstitute in 220 µL of Assay Buffer 8 and mix well by pipetting up and down. Aliquot Developer Solution V so that you have enough volume to perform the desired number of assays. Store at -20°C. Once reconstituted, use within two months. Keep on ice while in use.

OxiRed™ probe: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Keep at room temperature during the assay. Store at -20°C and **protect from light and moisture**. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

Stop Solution III: 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.

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

1. We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, 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. Be aware however that this might affect the stability of your samples, and the readings can be lower than expected.
2. Interferences:
   Reducing agents present in the sample will interfere with the assay. Keep DTT or β-mercaptoethanol below 5 µM.

Cells (adherent or suspension) samples:
1. Harvest the number of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).
2. Wash cells in cold PBS.
3. Resuspend cells in 200 µL of ice-cold Assay Buffer 8.
4. Homogenize tissue with a Dounce homogenizer sitting on ice.
5. Centrifuge sample for 15 minutes at 4°C at 10,000 x *g* using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Tissue Samples:
1. Harvest the amount of tissue necessary for each assay (initial recommendation = 100 mg).
2. Wash tissue in cold PBS.
3. Resuspend tissue in 200 µL ice-cold Assay Buffer 8.
4. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 - 15 passes.
5. Centrifuge samples for 15 minutes at 4°C at 10,000 x *g* using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Erythrocytes:

1. Harvest the amount of cells necessary for assay (initial recommendation: 200 µL),
2. Wash cells with cold PBS.
3. Resuspend cells in 200 µL of ice-cold Assay Buffer 8.
4. Homogenize cells with a Dounce homogenizer sitting on ice.
5. Centrifuge sample for 15 minutes at 4°C at 10,000 x *g* using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a new tube.
7. Keep on ice.

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

Liquid Samples (Plasma, Serum): Plasma and Serum 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.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- 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.

Prepare serial dilution of H_2O_2 Standard as follows:

1. 20 mM dilution: Add 5 µL of undiluted Hydrogen Peroxide Solution III Standard to 215 µL ddH_2O. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.
2. 1 mM dilution: Transfer 50 µL of 20 mM dilution to 950 µL ddH_2O. Gently pipette up and down and then mix well by inversion. **Use this to prepare standard curve for colorimetric assay.**
3. 0.1 mM dilution: Transfer 100 µL of 1 mM dilution to 900 µL ddH_2O. Gently pipette up and down and then mix well by inversion. **Use this to prepare standard curve for fluorometric assay.**

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

For colorimetric assay: Add 0, 2, 4, 6, 8, 10 µl of 1 mM H_2O_2 solution into 96-well plate and bring the final volume to 90 µl with Assay Buffer 8 to generate 0, 2, 4, 6, 8, 10 nmole/well H_2O_2 Standard **or** prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes (sufficient for duplicate standard curves):

For fluorometric assay: Add 0, 2, 4, 6, 8, 10 µl of 0.1 mM H_2O_2 solution into 96-well plate and bring the final volume to 90 µl with Assay Buffer 8 to generate 0, 0.2, 0.4, 0.6, 0.8, 1 nmole/well H_2O_2 Standard **or** prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes (sufficient for duplicate standard curves):

Standard #	Volume of 1mM or 0.1 mM Standard (µL) *	Assay Buffer 8 (µL)	Final volume standard in well (µL)	End H_2O_2 Amount (nmoles/well) Colorimetric Assay	End H_2O_2 Amount (nmoles/well) Fluorometric Assay
1	0	245	90	0	0
2	5	240	90	2	0.2
3	10	235	90	4	0.4
4	15	230	90	6	0.6
5	20	225	90	8	0.8
6	25	220	90	10	1.0

***NOTE: For colorimetric assay use 1mM standard, for fluorometric assay use 0.1 mM standard.**

4. Add 10 µL of Stop Solution III into each standard well.

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.

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

1. Set up Reaction wells:
- Standard wells = 100 µL standard dilutions. [90 µL Standard + 10 µL Stop Solution III].
- Positive Control = 1-5 µL Positive Control (adjust volume to 78 µL/well with Assay Buffer 8).
- Positive Control High Control (HC) = 1-5 µL Positive Control (adjust volume to 78 µL/well with Assay Buffer 8).
- Sample wells = 2-78 µL samples (adjust volume to 78 µL/well with Assay Buffer 8)
- Sample High Control (HC) wells = 2-78 µL samples (adjust volume to 78 µL/well with Assay Buffer 8).

2. HC Catalase inhibition (for both colorimetric and fluorometric assay):
- Add 10 µL Stop Solution III into each Sample HC and Positive control HC wells. Do not add to any other wells.
- Mix well and incubate at 25°C for 5 minutes to completely inhibit the catalase activity in the sample HC wells.

3. Catalase Reaction (for both colorimetric and fluorometric assay):
- **Colorimetric:** Add 12 µL of fresh 1 mM H_2O_2 solution (see under standard preparation guidelines) into each sample, sample HC, positive control, and positive control HC wells.
- **Fluorometric:** Add 12 µL of a reaction mix containing 1.5 µL of fresh 1 mM H_2O_2 solution and 10.5 µL Assay Buffer 8 into each sample, sample HC, positive control, and positive control HC wells. Prepare a master mix to ensure consistency using the following calculation:

X µL component x (Number reactions +1)

1. Mix well and Incubate reaction at 25°C for 30 minutes.
*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.

2. Add 10 µL of Stop Solution III into each sample and positive control well.
Note: Do not add Stop Solution III into sample HC or Positive Control HC wells. This was done in the catalase inhibition step.
Note: All wells should have a volume of 100 µL.

4. Developer Mix
Each well (standards, samples, and controls) requires 50 µL of Developer Mix as shown in the table below. To ensure consistency, use the table below to prepare a Master Mix of the appropriate Developer Mix for your assay using the following calculation: X µL component x (Number reactions +1)

Component	Colorimetric Assay Developer Mix (µL)	Fluorometric Assay Developer Mix (µL)
Assay Buffer 8	46	47.7
OxiRed™ probe	2	0.3
Developer Solution V	2	2

5. Mix Master Reaction Mix by inversion. Add 50 µL Developer Mix into each standard, sample, sample HC, positive control and positive control HC wells. Use a clean tip for each well.
6. Mix and incubate at 25°C for 10 minutes, protected from light.
7. Measure absorbance immediately on a microplate reader at OD 570 nm for Colorimetric assay or fluorescence at Ex/Em= 535/587 nm for Fluorometric assay.

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

Calculations:

- The catalase activity present in the sample is inversely proportional to the signal obtained. Samples producing signals greater than that of the highest standard have low amounts of catalase. For optimization, more concentrated samples or higher volumes of samples should be tested.
- Conversely, samples that give a reading very close to zero have high levels of catalase. These samples should be diluted to ensure the readings fall within the standard range.

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).

2. Average the duplicate reading for each standard and sample.
3. Calculate the equation of the standard curve using a linear regression and determine the slope.
4. Calculate ΔOD/ΔRFU signal in the sample as follows:

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

$$\Delta RFU = RFU_{HC} - RFU_{sample}$$

Where:

HC = the reading of the sample High Control

Sample = the reading of the sample.

5. Apply the ΔOD/ΔRFU to H_2O_2 Standard Curve (colorimetric or fluorometric as per assay) to get B nmoles of H_2O_2 decomposed by catalase during the 30 min reaction. ***Note**: The ΔOD/ΔRFU values should fall within the Standard Curve range.

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

$$Catalase\ activity = \frac{B}{30 \times V} \times D = \frac{nmol}{min \times mL} = mU/mL$$

Where:

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

30 = Catalase reaction time (minutes)

V = the pretreated sample volume added into the reaction well (in mL)

D = sample dilution factor (before adding to the plate)

1 Unit Catalase activity = amount of catalase that will decompose 1.0 µmol of H_2O_2 per minute at pH 4.5 at 25°C.

FAQs

Q. The concentration of H_2O_2 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_2O_2 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. A drop in signal should be observed between the Positive Control HC and Positive control well which will prove the enzyme is active. The positive control is provided to validate that the assay components are working, not to be used for comparison with samples. Please note that the more positive control is added to the wells, the lower the reading values will be.

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 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 H_2O_2? Catalase causes degradation of H_2O_2 while peroxidase uses H_2O_2 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.

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

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