ab118184 –

Catalase Specific Activity Assay Kit

Instructions for Use

For the measurement catalase (CAT) activity

This product is for research use only and is not intended for diagnostic use.

Version 5b Last Updated 29 June 2020

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1. Introduction

Principle: ab118184 is used to determine the relative specific activity (activity and quantity) of catalase in a sample. Capture antibodies specific for catalase are pre-coated in the microplate wells. The native enzyme is immunocaptured within the wells of the microplate; this removes all other enzymes. The assay buffer contains hydrogen peroxide which reacts with a substrate to make a luminescent product. Catalase functions rapidly to remove hydrogen peroxide from the solution and reduce the production of light. Therefore the light produced in each well is inversely proportional to the amount of catalase activity (Figure 1).

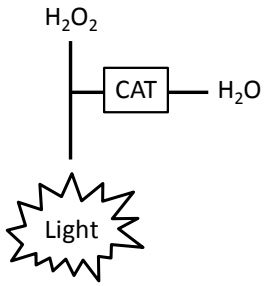


Figure 1. Scheme of reactions used in this kit. Abbreviations are as follows: catalase (CAT), Hydrogen peroxide (H_2O_2), water (H_2O).

After activity measurement the quantity of catalase is measured by adding to each well an anti-catalase primary detector antibody. After washing away unbound detector antibody, HRP-conjugated

labeled secondary antibody specific for the primary detector antibody is pipetted to the wells. The wells are again washed, an HRP substrate solution is added to the wells and color develops in proportion to the amount of catalase bound. The developing blue color is measured at 600 nm. Optionally the reaction can be stopped by adding hydrochloric acid which changes the color from blue to yellow and the intensity can be measured at 450 nm.

Background: Catalase is a heme containing peroxisomal homotetrameric enzyme that has a detoxification role by catalyzing the decomposition of the toxic cellular byproduct hydrogen peroxide (H_2O_2) to water and oxygen. Hydrogen peroxide is formed in the eukaryotic cell as a by-product of various oxidase and superoxide dismutase reactions. Hydrogen peroxide is metabolized by catalase and also glutathione peroxidase. Hydrogen peroxide is highly deleterious to the cell and its accumulation causes oxidation of cellular targets such as DNA, proteins, and lipids leading to mutagenesis and cell death. Therefore removal of the hydrogen peroxide from cells by catalase provides protection against oxidative damage. The highest levels of catalase in humans are found in the liver, kidney and erythrocytes. Defects in the catalase gene (CAT) are the cause of acatalasia (ACATLAS); also known as acatalasemia. This disease is characterized by absence of catalase activity in red cells and is often associated with ulcerating oral lesions.

This assay improves upon existing catalase activity assays by isolating catalase from within each sample, this allows -

- Sequential measurement of both activity and quantity of catalase in each well, the relationship between which is the specific activity.
- Removal of other competing H_2O_2 metabolizing enzymes such as glutathione peroxidase.
- Removal of H_2O_2 creating enzymes in a sample such as superoxide dismutase or other oxidases.

This assay also uses a novel luminescent detection method which allows the rapid and stable catalase activity to be measured. Therefore, unlike other methods, the reaction can be measured shortly after addition of substrate and need not be stopped but can be followed for a long period of time.

Limitations:

- FOR RESEARCH US ONLY. NOT FOR DIAGNOSTIC PROCEDURES.
- Use this kit before expiration date.
- Do not mix or substitute reagents from other lots or sources.
- If experimental samples generate values above the range of the control or normal sample standard curve, further dilute the samples with 1X Incubation buffer and repeat the assay.

- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

Technical Hints:

- To avoid cross contamination, change pipette tips between additions of each sample and between reagent additions. Also use separate clean, dry reservoirs for each reagent.
- Cover plate during incubation steps.
- Thorough and consistent wash technique is essential for proper assay performance. Wash buffer must be forcefully dispensed and completely removed from the wells by aspiration or decanting. Remove remaining wash buffer by inverting the plate and blotting on paper towels.

2. Assay Summary

Activity Determination

Prepare samples as instructed. Determine the protein concentration of extracts.

Equilibrate all reagents to room temperature.

Dilute sample to desired protein concentration in 1X Incubation Buffer. Add 100μL sample to each well used. Incubate 3 hours at room temp.

Aspirate and wash each well twice. Add 100 μL 2X Activity Solution to each well. Add 100 μL 2X H_2O_2 Solution to each well.

Pop bubbles and record immediately the luminescence in each well for 30 minutes.

Quantity Determination

Aspirate and wash each well once.

Add 100 μL of prepared detector antibody to each well. Incubate 1 hour at room temperature.

Aspirate and wash each well twice. Add 100 μL of HRP label to each well. Incubate 1 hour at room temperature.

Aspirate and wash each well three times. Add 100 μL HRP Development Solution to each well. Immediately begin recording the color development with elapsed time at 600 nm for 15 minutes. Alternatively add a Stop solution at a user-defined time and read at 450 nm.

3. Kit Contents

Sufficient materials are provided for 96 measurements in a microplate.

Item	Quantity
Extraction Buffer	15 mL
20X Buffer	20 mL
10X Blocking Solution	10 mL
Base Buffer	24 mL
200X Luminescent Reagent	0.2 mL
55X Coupler	0.5 mL
60X Hydrogen Peroxide (30%)	0.5 mL
HRP Development Solution	12 mL
10X Detector Antibody	1.5 mL
10X HRP Label	1.5 mL
96 well microplate (black)	1

4. Storage and Handling

All components are shipped cold. Store all components at 4°C. This kit is stable for 6 months from receipt.

5. Additional Materials Required

- Activity - Standard or fluorescence plate reader capable of luminometry.
- Quantity - Plate reader should also be able to measure in standard absorbance mode at 600nm or 450 nm.
- Purified human catalase (ab91026) can be used as a calibration standard in this assay if desired. This component is shipped on dry ice and should be stored at -80°C.
- Multichannel pipette (50 - 300 µL) and tips
- 15 mL and 1.5 mL tubes.
- Paper towels
- Deionized water

6. Reagent Preparation

6.1 Prepare 1X Wash Buffer by adding 20 mL 20X Buffer to 380 mL nanopure water. Mix throroughly.

6.2 Prepare 1X Incubation Buffer by adding 10 mL 10X Blocking Solution to 90 mL 1X Wash Buffer. Mix thoroughly.

6.3 Immediately prior to use prepare enough 2X Activity Solution. For an entire plate dispense 12 mL Base buffer into a clean tube. Add 0.455 mL 55X Coupler and 0.125 mL 200X Luminescent Reagent. Mix well.

6.4 Immediately prior to use prepare enough 2X Hydrogen Peroxide Solution. For an entire plate dispense 12 mL Base buffer into a clean tube. Add 0.417 mL 60X Hydrogen Peroxide. Mix well.

7. Test Sample Preparation

Note: Extraction buffer can be supplemented with PMSF and protease inhibitor cocktail prior to use. Supplements should be used according to manufacturer's instructions.

7.1 Cell lysates:

7.1.1 Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 10 minutes at 4^{o}C.

7.1.2 Rinse cells twice with PBS.

7.1.3 Solubilize cell pellet at 2x10^{7}/mL in Extraction Buffer.

7.1.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g, 4°C for 20 minutes. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

7.2 Tissue lysates:

7.2.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

7.2.2 Suspend the homogenate to 25 mg/mL in PBS.

7.2.3 Solubilize the homogenate by adding 4 volumes of Extraction Buffer to a sample protein concentration of 25 mg/mL.

7.2.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g, 4°C for 20 minutes. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

7.3 Sub-cellular organelle lysates e.g. peroxisomes:

7.3.1 Prepare the organelle sample by, for example, sub-cellular fractionation.

7.3.2 Pellet the sample.

7.3.3 Solubilize the pellet by adding 9 volumes Extraction Buffer.

7.3.4 Incubate on ice for 20 minutes. Centrifuge at 16,000 x g, 4°C for 20 minutes. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.

These test samples should be diluted to within the working range of the assay in 1X Incubation Buffer. As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Data Analysis.

8. Assay Procedure

Equilibrate all reagents and samples to room temperature before use. It is recommended all samples and standards be assayed in duplicate.

8.1 Activity Measurement

8.1.1 Prepare all reagents, and samples as directed in the previous sections.

8.1.2 Add 100 µL of each diluted sample per well. It is recommended to include a dilution series of a control (normal) sample as a reference. Also include a 1X Incubation Buffer as a zero standard.

8.1.3 Cover/seal the plate and incubate for 3 hours at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.

8.1.4 Aspirate each well and wash, repeat this once more for a total of **two** washes. Wash by aspirating or

decanting from wells then dispensing 300 μL 1X Wash Buffer into each well as described above. Complete removal of liquid at each step is essential for good performance. After the last wash, remove the remaining buffer by aspiration or decanting. Invert the plate and blot it against clean paper towels to remove excess liquid.

8.1.5 Gently add 100 μL 2X Activity Solution (step 6.3) to each well minimizing the production of bubbles.

8.1.6 Gently add 100 μL 2X Hydrogen Peroxide Solution (step 6.4) to each well minimizing the production of bubbles.

8.1.7 Pop any bubbles immediately and record luminescence in the microplate luminometer prepared as follows:

Mode:	Luminometer
Time:	Read for 30 minutes
Shaking:	Shake before reading

8.1.8 Record the data for analysis as described in section 9, **Data Analysis** - **Activity** below.

8.2 Quantity measurement

8.2.1 Immediately prior to use prepare sufficient (12 mL for the entire plate) 1X Detector Antibody in 1X Incubation buffer. Repeat the aspirate/wash procedure above.

8.2.2 Add 100 μL 1X Detector antibody to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.

8.2.3 Repeat the aspirate/wash procedure above.

8.2.4 Immediately prior to use prepare sufficient (12 mL for the entire plate) 1X HRP label in 1X Incubation buffer. Add 100 μL 1X HRP label to each well used. Cover/seal the plate and incubate for 1 hour at room temperature. If available use a plate shaker for all incubation steps at 300 rpm.

8.2.5 Repeat the aspirate/wash procedure above, however, performing a total of **three** washes.

8.2.6 Add 100 μL HRP Development Solution to each empty well and immediately begin recording the blue color development with elapsed time in the

microplate reader prepared with the following settings:

Mode:	Kinetic
Wavelength:	600 nM
Time:	up to 15 min.
Interval:	20 sec. - 1 min.
Shaking:	Shake between readings

Alternative– In place of a kinetic reading, at a ***user defined***, time record the endpoint OD data at (i) 600 nm or (ii) stop the reaction by adding 100 µL stop solution (1N HCl) to each well and record the OD at 450 nm.

8.2.7 Record the data for analysis as described in section 9, **Data Analysis** - **Quantity** below.

9. Data Analysis

Activity - Example data sets collected after a 10 minute read are shown below illustrating data analysis of catalase activity measurements in HepG2 cells (as an example human cell line), Figure 2. Alternatively purified catalase from human erythrocytes (ab91026) can be used as a standard in this assay if desired, Figure 3.

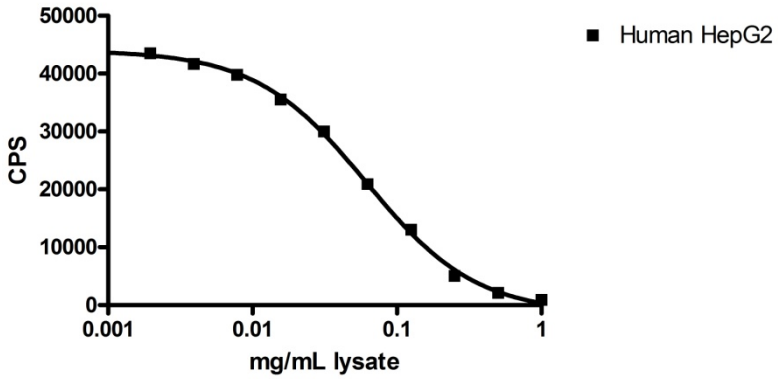


Figure 2. Example sample curves demonstrating the working range of the assay for a human and cultured cell line lysate (HepG2).

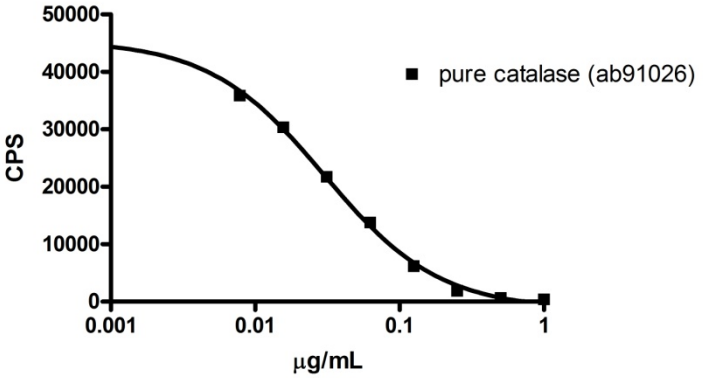


Figure 3. Example standard curve for the purified human catalase sample ab91026.

To do this average the duplicate control sample readings and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most luminometer software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic).

Unknown samples should be interpolated from a reference sample curves. This determined relative activity is the amount of reference sample required to generate the same amount of activity as the unknown sample and usually expressed as a per cent value.

ASSAY WORKING RANGE

This assay has been demonstrated with human, rat, and mouse liver and tissue homogenate samples as well as HepG2 whole cell lysate. Typical ranges for several sample types are described below. It is highly recommended to prepare multiple dilutions for each sample to ensure that each is in the working range of the assay.

Sample Type	Range
Cultured whole cell extracts (type dependant) e.g. HepG2	0.001 – 1 mg/mL
Tissue extract (liver)	0.001 – 1 mg/mL
Tissue extract (mouse)	0.001 – 1 mg/mL
Tissue extract (rat)	0.001 - 0.125 mg/mL
Purified human catalase	0.004 – 1 μg/mL

ACTIVITY REPRODUCIBILITY

Parameter	%CV
Intra (each n= 8)	5.6, 6.6, 9.2
Inter (n=4 days)	9.9

ACTIVITY LINEARITY OF DILUTION

Sample	% Expected
1:1	100
1:2	93
1:4	91

Quantity – Quantity data is analyzed in a very similar way to activity data. To do this average the duplicate control sample readings and plot against their concentrations. Draw the best smooth curve through these points to construct a standard curve. Most luminometer software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic), see Figures 4 and 5.

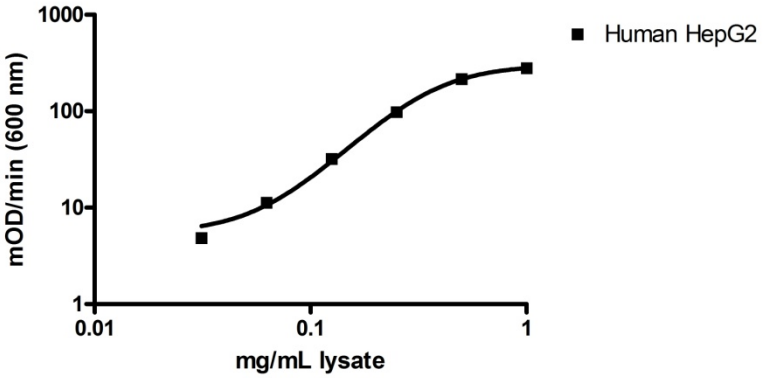


Figure 4. Example sample curves demonstrating the working range of the assay for a human cultured cell line lysate (HepG2).

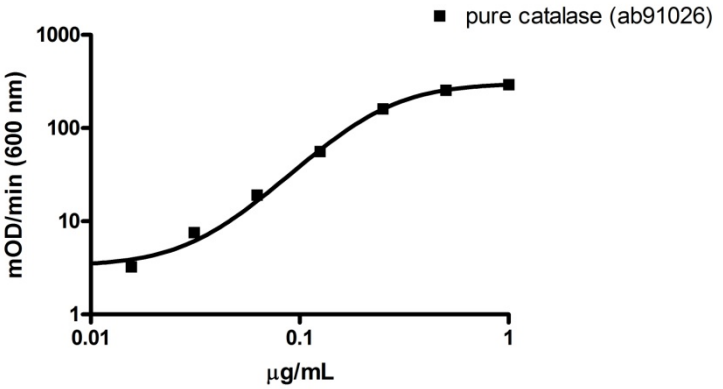


Figure 5. Example standard curve for the purified human catalase sample ab91026.

Unknown samples should be interpolated from these reference sample curves. This determined relative quantity is the amount of reference sample required to generate the same amount of signal as the unknown sample and usually expressed as a per cent value.

QUANTITY REPRODUCIBILITY

Parameter	%CV
Intra (each n= 8)	3.2, 5.2, 6.5
Inter (n=4 days)	2.3

QUANTITY LINEARITY OF DILUTION

Sample	% Expected
1:1	100
1:2	93
1:4	91
1:8	94
1:16	103
1:32	101

Relative specific activity – this assay determines the activity and quantity of catalase in a sample relative to either a control sample or purified human catalase. The relationship between the determined activity and the determined quantity can be expressed as a ratio or graphed as shown below (Figure 6). Changes in the relationship of activity to quantity indicate an increase or decrease in the activity of individual catalase molecules, for example due to modifications resulting from damage or regulation.

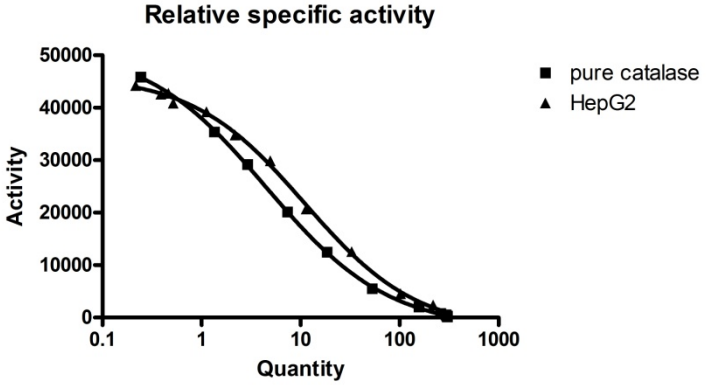
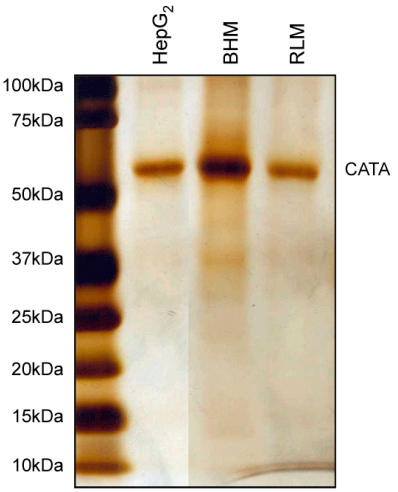


Figure 6. Example relative specific activity – comparing the activity and quantity of purified erythrocyte catalase (ab91026) and catalase from human hepg2 lysate. The HepG2 (▲) sample is determined to have a higher specific activity rate.

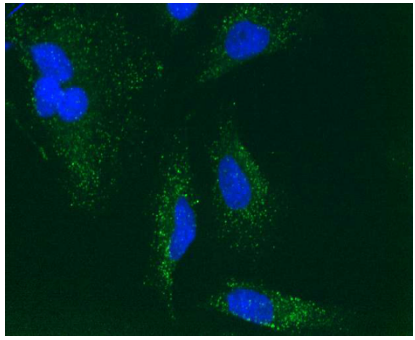
SPECIFICITY

Species– human, rat, and mouse reactive. Others untested.

The anti-catalase antibody used as a detector antibody in this kit was ab110292. This antibody was generated by immunization of rat liver proteins. The resulting monoclonal mouse antibody isolates, by immunoprecipitation, a single catalase band to purity from a number of species (shown below). The immunoprecipitate was confirmed to be catalase by mass spectrometry. This antibody is cross reactive in immunofluorescence microcopy and labels a peroxisomal intracellular pattern (shown below)



Immunoprecipitation with ab110292.



Immunofluorecent labeling of peroxisomes with ab110292.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)