MA-0143

Catalase Activity Assay

(100 wells, Colorimetric, OD 570 nm, Store at 4°C)

Introduction:

Hydrogen peroxide is a common metabolic byproduct of many cellular processes. A potent oxidant, it's toxic due to its ability to damage cell structures, proteins and DNA. Catalase evolved to remove hydrogen peroxide and is one of the most efficient enzymes with turnover numbers approaching $3x10^6$ s^{-1}. It acts in maintaining a level of ROS in cells necessary for normal physiological functions without allowing harm from excessive amounts. AkrivisBio's Catalase Activity Assay is a simple, sensitive way to measure catalase activity in a range of biological samples. In the assay, any hydrogen peroxide not consumed by catalase is utilized by peroxidase to convert ADHP to resorufin with strong absorbance and fluorescence.

Assay Principle:

1 – Hydrogen peroxide is added to the assay mix, allowing catalase to break it down
2 – After a time, peroxidase is added to the assay along with ADHP and utilizes any hydrogen peroxide remaining to form resorufin.
3 – The difference in H_2O_2 content between test wells and a baseline well with no catalase defines the amount of catalase activity present.

Assay Components:

Assay Buffer	25 ml	NM	MA-0143-A
ADHP Solution	200 µl	Red	MA-0143-B
Peroxidase	lyoph	Green	MA-0143-C
H_2O_2	25 µl	Yellow	MA-0143-D
Inhibitor/Stop Solution	1 ml	White	MA-0143-E
Catalase Pos Control	2 µl	Blue	MA-0143-F

Storage and Handling Considerations:

Store at 4°C. Centrifuge all vials briefly and warm all components to room temperature before use.
Assay Buffer: Ready to use as supplied.
ADHP Solution: Ready to use as supplied**.** Verify DMSO is melted before use. Store at 4°C.
Peroxidase: Reconstitute using 220 µl Assay Buffer. Store at 4°C.
Inhibitor/Stop Solution: Ready to use as supplied.
Positive Control: Add 200 µl Assay Buffer to Positive Control. Divide into aliquots and store at -20°C.

Assay Protocol:

1. H_2O_2 Standard Curve:

Absorbance-based assay: Transfer 10 µl of H_2O_2 to 870 µl DI H_2O giving a 10 mM H_2O_2 solution. Dilute further, transferring 20 µl into 480 µl DI H_2O, giving a 0.4 mM solution. Save the remaining 10 mM solution on ice to make more 0.4 mM H_2O_2, if necessary. Transfer 0 – 5 – 10 – 15 – 20 - 25 µl to a series of wells in a 96-well plate, giving 0, 2, 4, 6, 8, 10 nmol of the H_2O_2 Standard. Bring all well volumes to 100 µl with Assay Buffer.

Fluorescence-based assay: Dilute the H_2O_2 as for the absorbance-based assay, then dilute another 10X to 40 µM then transfer 0 – 5 – 10 – 15 – 20 - 25 µl to a series of wells in a 96-well plate giving 0, 200, 400, 600, 800, 1000 pmoles per well. Adjust all wells to 100 µl.

Note: Reducing agents present in samples break down H_2O_2. o only use samples that do not contain TCEP, DTT or β-ME.

2. Test Samples:

Homogenize 10 mg of tissue, or 10^6 cells in 200 µl of cold Assay Buffer. Centrifuge (16,000 x g, 4°C, 10 min.). Transfer supernatant to a fresh tube on ice. Liquid samples can be used directly. Transfer 2 - 50 µl of each sample to a 96-well plate and adjust all well volumes to 100 µl with Assay Buffer. Enzymes other than catalase can utilize H_2O_2. If such enzyme activity is present, run the test well in duplicate, using the paired well as background control by adding 10 µl of Inhibitor/Stop Solution to the paired well only (containing NaN_3) to inhibit any catalase present. Mix and incubate for 5 min to inhibit catalase activity.

3. Positive Control: Dilute the positive control by adding 10 µl to 90 µl of Assay Buffer. Add 1 – 5 µl of the positive control to a well and bring the volume to 100 µl.

4. Initiate Catalase Activity:

Add 25 µl of the 0.4 mM H_2O_2 (prepared for the standard curve) into each well (test wells, background controls and positive control) to start the reaction. Add 25 µl of the 0.4 mM H_2O_2 to an empty well and bring the volume to 100 µl with Assay Buffer. This serves as 0 catalase activity (high control) well showing the initial H_2O_2 concentration before reaction. Incubate the plate at 25°C for 3-5 min, then add 10 µl Stop Solution to each test and Positive Control well. For samples with very low catalase activity the incubation period should be increased appropriately.

5. Developer:

Prepare sufficient developer for the total number of wells being run to determine remaining H_2O_2**.** Each test, background control, positive control, high control and standard curve well will require 50 µl of developer containing:

Assay Buffer	46 µl
ADHP Solution	2 µl
Peroxidase	2 µl

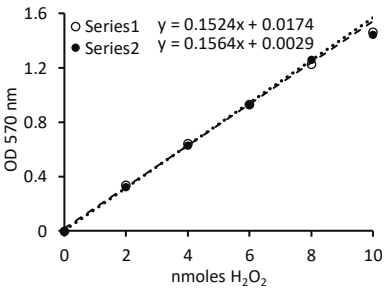
Add 50 µl of the Developer to all wells. Place the plate on a shaker at 25°C for 10minutes.

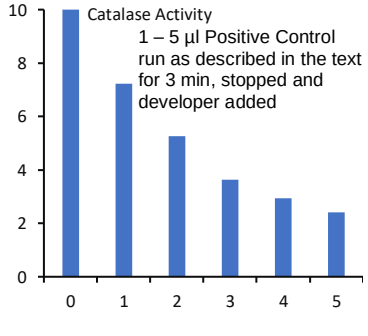
6. Measurement:

Read the absorbance of all wells at 570 nm in a plate reader.

7. Typical results:

nmole Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0441	0.0451	0	0
2	0.3799	0.3669	0.3358	0.3218
4	0.6870	0.6746	0.6429	0.6295
6	0.9726	0.9775	0.9285	0.9324
8	1.2716	1.3037	1.2275	1.2586
10	1.5047	1.4904	1.4606	1.4453





8. Calculation: Catalase activity is simply $\Delta A/\Delta t = A_{\text{High Control}} - A_{\text{sample}}$ at time t. Subtract the zero standard from all standards and plot the H_2O_2 Standard Curve. Determine the slope of the standard curve. Divide the ΔA determined for the test samples by the slope of the standard curve to convert to nmoles. Divide by Δt to get nmoles per minute (mU) per well. Divide by the volume of sample added per well to get mU/µl of sample. Multiply by total volume of supernatant recovered in step 2 above to get total mU of activity per sample, then divide by mg tissue or # of cells or other amount of sample to get mU per mg of tissue (or per # of cells, etc.)

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