MA-0145

Hydrogen Peroxide Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Introduction:

Hydrogen peroxide is important in cellular metabolism due to its role as a reactive oxygen species. Although considered a harmful byproduct of metabolism, low levels of H_2O_2 are essential for some physiological processes. H_2O_2 acts as a signaling molecule, participating in signaling pathways that regulate responses to stress, growth, and immune functions. $H_2O_2$is produced during aerobic respiration as a byproduct of cellular energy production. Mitochondria are a major source of H_2O_2 production. The controlled generation of H_2O_2 serves as an intermediary in cellular signaling, assisting in the activation of various enzymes involved in key metabolic pathways. H_2O_2 plays a role in the immune response, acting as a defense mechanism against invading pathogens. Immune cells use hydrogen peroxide to neutralize bacteria and viruses, highlighting its role in combating infections. AkrivisBio's Hydrogen Peroxide Assay is a simple, sensitive means of measuring peroxide in a variety of samples. It can be used in either absorbance or fluorescence-based measurements.

Assay Principle:

Peroxidase utilizes hydrogen peroxide to oxidize ADHP to resorufin, resulting in intense color (570 nm) and fluorescence (excitation 535 nm, emission 587 nm).

Assay Components:

Assay Buffer	25 ml	WM	MA-0145A
ADHP Solution	0.2 ml	Red	MA-0145B
Peroxidase	lyoph	Green	MA-0145C
H_2O_2 Standard	0.1 ml	Yellow	MA-0145D

Storage and Handling Considerations:

Store the unopened assay at -20°C. Centrifuge all vials briefly before opening.
Assay Buffer: Ready to use as supplied. Bring to room temperature before using.
ADHP Solution: Ready to use as supplied. Bring to room temperature before using.
HRP: Reconstitute with 220 µl of Assay Buffer. Store at -20°C. If you anticipate using the assay repeatedly over a period of time, divide the enzyme into several portions to avoid repeated freeze/thaw cycles.

Assay Protocol:

1. Standard Curve:
Absorbance-based assay – Transfer 10 µl the H_2O_2 Standard to 870 µl of DI H_2O. Dilute further by transferring 10 µl to 990 µl of DI H_2O giving a 100 µM working solution. Transfer 0 – 10 – 20 – 30 – 40 – 50 µl to a series of wells in a 96-well plate giving 0, 1, 2, 3, 4, 5 nmoles of H_2O_2. Adjust all well volumes to 50 µl with Assay Buffer.
Fluorescence-based assay – Prepare the standard as for the absorbance-based assay, then dilute further by transferring 50 µl to 450 µl of DI H_2O. giving a 10 µM standard working solution. Transfer 0 – 10 – 20 – 30 – 40 – 50 µl to a series of wells in a 96-well plate giving 0, 100, 200, 300, 400, 500 pmoles of H_2O_2. Adjust all well volumes to 50 µl with Assay Buffer.

2. Samples:
Liquid samples should be kept cold and filtered through a 3 or 10 kDa centrifugal filter. Peroxidase activity in samples can destroy any peroxide present so samples should be prepared as rapidly as possible. Alternatively, samples can be treated with protein precipitants such as TCA or sulfosalicylic acid and filtered or centrifuged to remove protein. In the absence of stabilizer, hydrogen peroxide is rather unstable so samples should be assayed as soon as possible. If they can't be immediately tested, store at -80°C until they can. Add up to 50 µl of test samples to wells and adjust all well volumes to 50 µl with Assay Buffer.

3. Initiate Reaction: Each Standard and Test Sample well will require 50 µl of Reaction Mix. Prepare sufficient Mix for the total number of wells to be analyzed, using (per well):

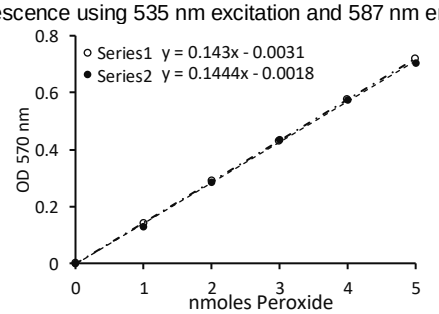
	Absorbance- based Assay	**Fluorescence-based Assay**
Assay Buffer	46 µl	47 µl
ADHP Solution	2 µl	1 µl
Peroxidase	2 µl	2 µl

Add 50 µl of the Reaction Mix to all Standard and Test sample wells.

4. Measurement: Measure the absorbance at 570 nm or fluorescence using 535 nm excitation and 587 nm emission in end-point mode.

5. Typical Results:

	Standard Raw Values		Background Corrected Values	
nmole Standard	OD			
0	0.0437	0.0481	0	0
1	0.1826	0.1776	0.1389	0.1295
2	0.3335	0.3330	0.2898	0.2849
3	0.4735	0.4823	0.4298	0.4342
4	0.6202	0.6207	0.5765	0.5726
5	0.7639	0.7534	0.7202	0.7053



5. Calculation: Subtract the zero-standard reading from all other standard and test sample readings. Plot the Standard Curve and determine the slope of the Standard Curve. This defines the system sensitivity of the assay. Divide the background corrected test sample readings by the slope of the standard curve to get the amount of peroxide in the wells. To convert back to peroxide content of the original test samples:

A – Divide the amount of peroxide in the wells by the volume of sample added to each well = amount of peroxide per µl of sample

B – Multiply the amount of peroxide per µl of sample by the total volume of each sample in step 2 above = total amount of peroxide in the sample.

C – If the sample was derived from a tissue sample or cell culture, divide the total amount of peroxide in the sample by the mg of tissue or by the # of cells sampled to get amount of peroxide per mg of tissue or per # of cells.

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