Catalase Activity Colorimetric/Fluorometric Assay Kit

(Catalog #BN00986; 100 reactions; Store kit at 4°C)

I. Introduction:

Catalase (EC 1.11.1.6) is a ubiquitous antioxidant enzyme that is present in nearly all living organisms. It functions to catalyze the decomposition of hydrogen peroxide (H_20_2) to water and oxygen. Assay Genie's Catalase Assay Kit provides a highly sensitive, simple, direct and HTS-ready assay for measuring Catalase activity in biological samples. In the assay, catalase first reacts with H_2O_2 to produce water and oxygen, the unconverted H^2O^2 reacts with GenieRed 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 samples.

II. Kit Contents:

Components	BN00986	Cap Code	Part Number
Catalase Assay Buffer	25 ml	NM	BN00986-1
GenieRed Probe (in DMSO)	200 µl	Red	BN00986-2
HRP (lyophilized)	1 vial	Green	BN00986-3
H_2O_2 (0.88M)	25 µl	Yellow	BN00986-4
Stop Solution	1 ml	White	BN00986-5
Catalase Positive Control	2 µl	Blue	BN00986-6

III. Storage and Handling:

Store kit at 4°C, protect from light. Warm the assay buffer to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

GenieRed Probe: Briefly warm to completely melt the DMSO solution. Store at 4° C, protected from light. Use within two months.

HRP: Dissolve with 220 µl Assay Buffer. Store at 4° C. Use within two months.

Positive Control Solution: Add 500 µl Assay Buffer to Positive Control. Aliquot and store at -20°C. Diluted Positive Control solution is stable for 2-3 days at 4° C & for 2 months at -20°C.

Note: Keep samples, HRP and Catalase on ice while in use.

V. Catalase Activity Assay:

1. Sample and Positive Control Preparations:

Homogenize 0.1 gram tissues, or 10^6 Cells, or 0.2 ml Erythrocytes on ice in 0.2 ml cold Assay Buffer; Centrifuge at 10,000 x g for 15 min at 4° C; Collect the supernatant for assay, keep on ice. Liquid samples can be tested directly. Store samples at -80° C to assay later.

Add 2 - 78 µl of samples or 1 - 5 µl Positive Control Solution into each well, and adjust volume to total 78 µl with Assay Buffer. Prepare sample High Control (HC) with the same amount of sample in separate wells then bring total volume to 78 µl with Assay Buffer. Add 10 µl of Stop Solution into the sample HC, mix and incubate at 25° C for 5 min to completely inhibit the catalase activity in samples as High Control. For unknown samples, we suggest testing several doses of your sample to ensure the readings are within the linear range.

Reducing agents in samples interfere with the assay. Keep DTT or β-ME below 5 µM.

2. H_2O_2 Standard Curve:

Dilute 5 µl of 0.88M H_2O_2 into 215 µl dH_2O to generate 20 mM H_2O_2, then take 50 µl of the 20 mM H_2O_2 and dilute into 0.95 ml dH_2O to generate 1 mM H_2O_2. Add 0, 2, 4, 6, 8, 10 µl of 1 mM H_2O_2 solution into 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well H_2O_2 standard. Bring the final volume to 90 µl with Assay Buffer. Add 10 µl Stop Solution into each well. For the fluorometric assay, dilute the standard H_2O_2 10-fold for the standard curve (0-1 nmol range).

Note: Diluted H_2O_2 is unstable, prepare fresh dilution each time.

3. Catalase Reaction:

Add 12 µl fresh 1 mM H_2O_2 into each well (samples, positive control, and sample HC) to start the reaction, incubate at 25° C for 30 min, and then add 10 µl Stop Solution into each sample well (Sample, Positive Control; *do not add Stop Solution to the HC*) to stop the reaction (Note: High Control and standard curve wells already contain Stop Solution).

4. Develop Mix:

Mix enough reagents for the number of assays to be performed. For each well prepare a 50 µl Developer Mix containing:

46 µl Assay Buffer
2 µl GenieRed Probe
2 µl HRP solution

Add 50 µl of the Developer Mix to each test samples, controls, and standards. Mix well and incubate at 25° C for 10 min. Measure OD 570 nm in a plate reader. **Note:** For low amounts of catalase, you can either increase the incubation time prior to adding the Stop Solution or use the fluorometric method. For the fluorometric method, decrease the 1 mM H_2O_2 amount to1.5 µl and GenieRed Probe to 0.3 µl in the reaction; compensate the volume with Assay Buffer.

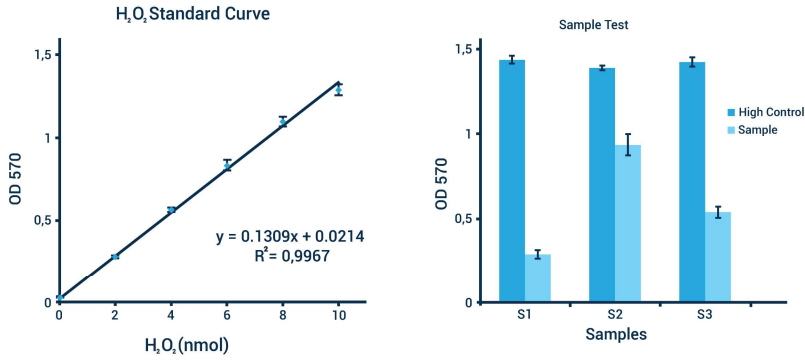
6. Calculation:

Signal change by catalase in sample is $\Delta A = A_{HC} - A_{sample}$. A_{HC} is the reading of sample High Control, A_{Sample} is the reading of sample in 30 min. Plot the H_2O_2 Standard Curve. Apply the ΔA to the H_2O_2 standard curve to get B nmol of H_2O_2 decomposed by catalase in 30 min reaction. Catalase activity can be calculated:

$$\textbf{Catalase Activity} = \frac{B}{30 \times V} \times \textbf{Sample Dilution Factor} = \textbf{nmol/min/ml} = \textbf{mU/mL}$$

Where: **B** is the decomposed H_2O_2 amount from H_2O_2 Standard Curve (in nmol).
V is the pretreated sample volume added into the reaction well (in ml).
30 is the reaction time 30 min.

Unit definition: One unit of catalase is the amount of catalase that decomposes 1.0 µmol of H_2O_2 per min at pH 4.5 at



FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		