ABS_Bio™ Paraoxonase Activity Detection Kit

(Cat# K214-100; 100 assays; store kit at -20°C)

Introduction

Serum paraoxonase (PON1) is a calcium-dependent esterase associated exclusively with HDL. PON1 exhibit a range of important activities, including drug metabolism and detoxification of organophosphates such as nerve agents. Serum PON1 activity may also be important in avoidance of organophosphate toxicity in industry. PON1 is a mammalian enzyme with hydrolase activity toward multiple substrates.

The ABS_Bio™ Paraoxonase Activity Detection Kit provides a simple, sensitive, one-step colorimetric assay to detect Paraoxonase (PON1) activity in a variety of samples. In this assay, PON1 hydrolyzes the specific substrate paraoxon to release the p-nitrophenol. The yellow product absorption at 405 nm is directly proportional to the PON1 activity in the sample. The kit is supplied with sufficient reagents for 100 tests in 96-well plate assay, linear detection activity range of 0.05-50 U/L. It could easily be modified for use in 384-well assay and high-throughput assay.

Kit Components (100 tests)

Assay Buffer:	12 mL	p-Nitrophenol Standard:	0.5 mL	Substrate:	0.12 mL	Stop Solution:	10 mL
Normal rat serum:	0.1 mL						

Storage and Handling: Store kit at -20°C. Shelf Life: 6 months after receipt. Warm up Reagents to room temperature before use.

Protocol

1. Sample preparation

Serum, Plasma, other body fluid, or cell culture supernatant can be measured directly by a series of dilutions of the sample to ensure the readings are within the standard curve range. Homogenize Cell (2×10^6) or tissue (20 mg) sample in 200 µL cold PBS. Centrifuge to collect the supernatant. It is recommended with all sample types to assay immediately or aliquot and store the samples at -80°C.
Transfer 20 µL sample (optional: series dilute normal rat serum as positive control) into the 96-well clear flat bottom plate in duplicate.

2. Standard Preparation

Transfer 20 µL of 10 mM p-Nitrophenol standard into 180 µL dH_2O to generate a 1 mM p-Nitrophenol standard, then use the following table to generate 1000, 600, 300, 100 and 0 µM p-Nitrophenol standards.

1mM p-Nitrophenol std(µL)	dH_2O(µL)	final p-Nitrophenol concentration (µM)
50	0	1000
30	20	600
15	35	300
5	45	100
0	50	0

Transfer 20 µL of appropriate standards into the 96-well plate in duplicate. The blank control contains dH_2O only.

3. Reaction

Prepare enough working reagent by mixing 80 µL assay buffer and 1 µL substrate for each reaction (samples, standards & positive control).
Note:Substrate is toxic liquid, should be handled in an air-extraction fumehood, waste disposal and pipettes should be treated with 2M NaOH.
Transfer 80 µL prepared working reagent into each reaction well. Note: For colored sample, please directly add 80 µL assay buffer into 20 µL of sample as sample blank (omit substrate). Tap plate to mix well.

4. Measurement

Read kinetic OD405 nm (400-410 nm) every minute on the plate reader for 30 minutes. Since the activity of the enzyme increases with time, it is recommended to read the plate at several time points (e.g t_1 and t_2 at 0 and 30 minutes). Then choose the data in the reaction linear range. Option (endpoint mode): incubate 30 minutes at room temperature, then add 50 µL stop solution into each reaction well to stop the reaction. Tap plate to mix well, then Read OD405 nm for 10 min.

5. Calculation

Average the duplicate OD405 nm reading for standard. Subtract the average OD of the blank (OD_{blank}) from the average OD of the standards and sample, then plot the standard result ($\Delta OD_{std} = OD_{30\ std} - OD_{blank}$) versus the p-Nitrophenol concentration to get p-Nitrophenol standard curve. Calculate sample results ($\Delta OD_{sample} = OD_{30\ sampe} - OD_{0\ sample}$), and determine sample's PON1 generated p-Nitrophenol (µM) from p-Nitrophenol standard curve. Note: if has sample blank, the sample blank reading must be subtracted from sample readings.

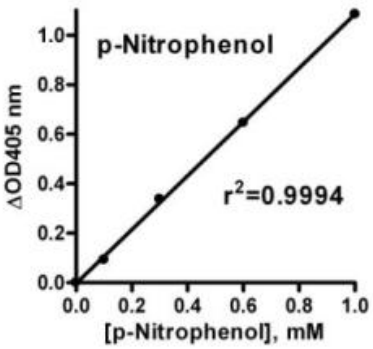
[p-Nitrophenol] = ΔOD_{sample} /Slope (µM)

Calculate the PON1 activity of samples as equation.
[Paraoxonase activity]= µM p-Nitrophenol / *t* * *n* (U/L) ***t* is the reaction time (t_2-t_1 minutes), *n* is the sample dilution factor.**

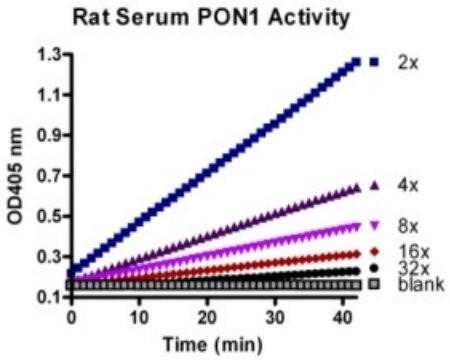
Unit Definition: One unit of activity is defined as the amount of enzyme that catalyzes hydrolysis of 1 µmol of substrate at 25 °C.

Note: We recommend to choose the period of linear range within the standard curve to calculate the PON1 activity of the samples. If sample PON1 activity over the linear range, either use a shorter reaction time or dilute samples in water and repeat the assay. For samples with lower PON1 activity, the incubation time can be extended or incubate at 37°C.

Typical Standard Curve



p-Nitrophenol standard in 96 wells-plate assay.



Rat Serum PON1 activity in 96 wells-plate assay.

Always run your own standard curves for calculation of results.

Sensitivity and Limit of Detection

The Limit of Detection was determined as 0.05 U/L, and linear detection range up to 50 U/L in 96-well plate colorimetric assay for 30 min. Samples with values above linear range should be dilute with dH_2O or assay buffer, re-assayed, and multiplied by dilution factor.

Interferences

The following chemicals are known to affect the enzyme activity and should be avoided during the assay. EDTA, Citrate (0.2 mM) and Zinc chloride (0.015 mM).

References

Charlton-Menys, V. et al. 2006, Clinical Chemistry. 52:453-457
Golmanesh, L. et al. 2008, J. Biochem. Biophys. Methods 70:1037–1042