Version 2b, Last updated 23 June 2025

ab241044 Paraoxonase 1 Activity Assay Kit

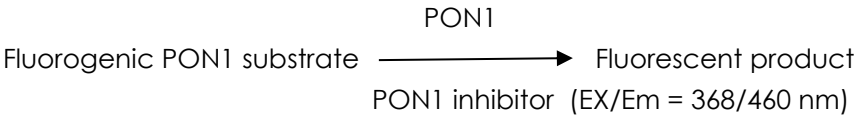
For the measurement of Paraoxonase 1 activity in human and animal plasma and serum.

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

Table of Contents

1. Overview

Paraoxonase 1 Activity Assay Kit (ab241044) enables rapid measurement of Paraoxonase 1 (PON1) activity, utilizing a fluorogenic substrate that is converted into a highly fluorescent product (Ex/Em = 368/460 nm). This ensures dramatically greater sensitivity than UV or colorimetric assays and eliminates the need for dangerous toxic substrates. A selective PON1 inhibitor is provided for verification of PON1 specific activity. The assay is simple to perform, high-throughput adaptable and can detect a minimum of 2.0 µU paraoxonase activity with a sample volume of 5 µL.



Prepare Standards, Reaction Mix, Inhibitor Mix, Background Mix, Positive Control and Positive Control + Inhibitor Mix.

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Add Samples to Test Mix and Inhibitor Mix. Add Positive Control to Positive Control Mix and Positive Control + Inhibitor Mix.

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Incubate plate for 10 minutes at 37°C.

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Add PON1 Substrate to all wells (apart from Standards).

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Immediately begin measuring the fluorescence at Ex/Em = 368/460 nm in kinetic mode for 60 minutes at 37°C. Standards may be read in end-point mode.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Paraoxonase Assay Buffer	50 mL	-20°C	-20°C
Fluorescence Standard III	1 vial	-20°C	-20°C
PON1 Inhibitor	1 vial	-20°C	-20°C
PON1 Substrate	1 vial	-20°C	-20°C
Paraoxonase Positive Control	1 vial	-20°C	-80°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multiwell fluorescence microplate reader capable of reading at Ex/Em = 368/460 nm
- Precision multi-channel pipette and reagent reservoir
- Anhydrous (reagent grade) DMSO
- Black 96-well plates with flat bottom

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Paraoxonase Assay Buffer

Ready to use as supplied. Allow to warm to room temperature before use.

5.2 Fluorescence Standard III

Reconstitute with 55 µL of DMSO to yield a 5 mM solution. Store at -20°C, stable for 3 freeze/thaw cycles.

5.3 PON1 Inhibitor

Reconstitute with 110 µL of DMSO and vortex to yield a 50 mM stock solution. To prepare a 2 mM working solution (10X final concentration), add 40 µL of the 50 mM stock solution to 960 µL of dH_2O. The 2 mM working solution should be stored at -20°C and is stable for 3 freeze/thaw cycles.

5.4 PON1 Substrate

Reconstitute with 44 µL of DMSO to obtain a 250X stock solution. Store at -20°C, stable for 3 freeze/thaw cycles.

5.5 Paraoxonase Positive Control

Reconstitute with 110 µL of Paraoxonase Assay Buffer and mix thoroughly. Aliquot and store at -80°C, avoid repeated freeze/thaw cycles.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

1. Dilute the Fluorescence Standard III by adding 10 µL of the 5 mM stock to 990 µL Paraoxonase Assay Buffer to obtain a 50 pmol/µL Standard solution.
2. Add 0, 2, 4, 6, 8, 12, 16 and 20 µL of the 50 pmol/µL solution into a series of wells in a black 96-well plate and adjust the volume of each well to 100 µL with Paraoxonase Assay Buffer, yielding 0, 100, 200, 300, 400, 600, 800 and 1000 pmol/well Fluorescence Standard III.

Standard#	50 pmol/ µL Fluorescence Standard III (µL)	Paraoxonase Assay Buffer (µL)	Final volume standard in well (µL)	Fluorescence Standard III in well (pmol/well)
1	0	100	100	0
2	2	98	100	100
3	4	96	100	200
4	6	94	100	300
5	8	92	100	400
6	10	90	100	500
7	12	88	100	600
8	16	84	100	800
9	20	80	100	1000

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

We recommend that you use fresh samples for the most reproducible assay.

7.1 Plasma or serum:

1. Collect plasma or serum samples by standard methods (keep on ice for immediate use or aliquot and store at -80°C for future experiments).
2. For human samples, we recommend adding 5 µl of undiluted serum/plasma per reaction, although volumes of 2-10 µl per reaction may be used.

ΔNote: As PON1 is a strongly Ca^{2+} -dependent enzyme, heparinized plasma samples should be used—plasma specimens collected with EDTA or other Ca^{2+} -chelating anticoagulants may exhibit reduced PON1 activity.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

8.1 Reaction wells set up:

- Standard wells = 100 µL standard dilutions.
- For other wells, refer to the table below (Section 8.2).

8.2 Paraoxonase assay mixes:

1. Prepare 80 µL of Test Mix, Inhibitor Mix, Background Mix (BC), Positive Control Mix (PC) and Positive Control + Inhibitor Mix for each reaction. Prepare a master mix to ensure consistency.

Component	Test Mix (µL)	Inhibitor Mix (µL)	Background Mix (µL)	Positive Control Mix (µL)	Positive control + Inhibitor Mix (µL)
Sample	2-10	2-10	-	-	-
Paraoxonsae Positive Control	-	-	-	10	10
PON1 Inhibitor	-	10	-	-	10
Paraoxonase Assay Buffer	to 80 µL	to 80 µL	80	70	60

2. Preincubate the plate for 10 minutes at 37°C to pre-warm samples and to allow the inhibitor to interact with sample PON1. During the preincubation, prepare a 5X concentrated PON1 Substrate solution by diluting the reconstituted 250X PON1 Substrate stock solution at a 1:50 ratio. Prepare 20 µL of 5X PON1 Substrate solution for each reaction to be performed (for example, for 10 wells, mix 4 µL of 250X PON1 Substrate stock with 196 µL Paraoxonase Assay Buffer).

3. Start the reaction by adding 20 µL of the 5X PON1 Substrate solution to each reaction well using a multichannel pipette, yielding a final volume of 100 µL/well.

ΔNote: Do not add PON1 Substrate solution to the standard curve wells.

8.3 Measurement:

Immediately (within 1 minute) begin measuring the fluorescence at Ex/Em = 368/460 nm in kinetic mode for 60 minutes at 37°C. We strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the sample.

Δ**Note:** The standard curve wells may be read in endpoint mode (Ex/Em = 368/460 nm).

9. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard, control and sample.
2. For the fluorescence standard curve, subtract the zero standard (0 pmol/well) reading from all of the standard readings, plot the background-subtracted values and calculate the slope of the standard curve.
3. For the reaction wells (including background control), choose two time points (t_1 and t_2) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points (RFU_1 and RFU_2) and determine the change in fluorescence over the time interval: $\Delta F = RFU_2 - RFU1$.
4. Calculate specific fluorescence (C_S) by subtracting the background control from each sample: $C_S = \Delta F_S - \Delta F_{BC}$.
5. Paraoxonase activity is obtained by applying the C_S values to the fluorescence standard curve to get B pmol of substrate metabolized during the reaction time.

$$PON1\ activity\ (A) = \frac{B}{(\Delta T * V)} * D$$

Where:

A = Paraoxonase activity (pmol/minute/mL = µU/mL).

B = amount of metabolite produced as calculated from standard curve (pmol).

ΔT = linear phase reaction time $t_2 - t_1$ (minutes).

V = sample volume added in the sample wells (mL).

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

10. Typical Data

Data provided **for demonstration purposes only**.

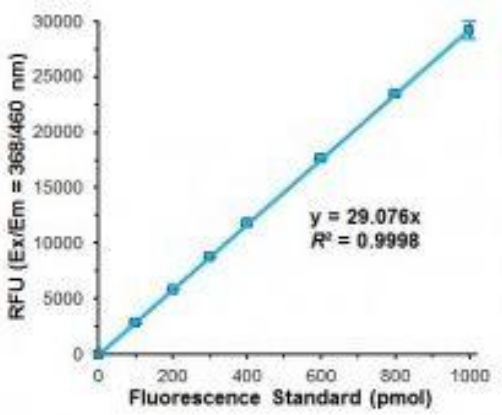

Figure 1. Fluorescence standard curve.

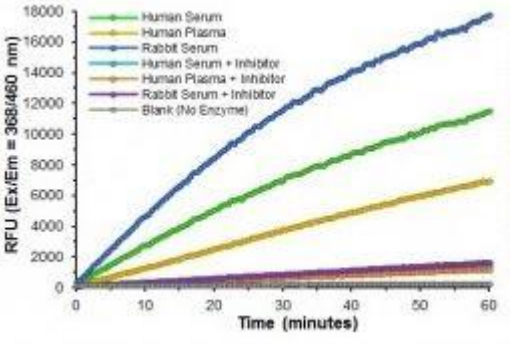

Figure 2. Reaction kinetics of PON1 Substrate metabolism in donor-pooled human serum (5 µl), donor-pooled human plasma (5 µL) and rabbit serum (2.5 µL) in the presence and absence of 200 µM of the selective PON1 inhibitor 2- hydroxyquinoline (no inhibitor conditions contained 0.4% DMSO as a solvent control).

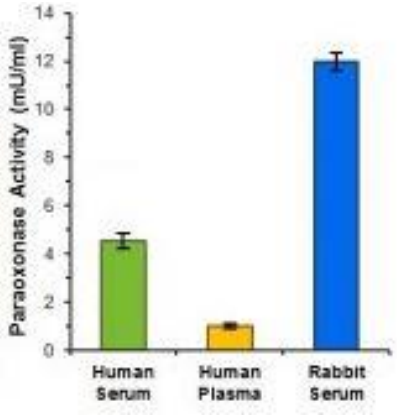

Figure 3. Quantification of PON1 activity in serum/plasma samples (mean ± SEM of four independent replicates).

11. Notes

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)