CREATIVE BIOMART®
Assay Kit

Paraoxonase 1 (PON1) Activity Assay Kit (Fluorometric)

Product Information

Cat.No. Kit-1109

Product Overview

Paraoxonase 1 Activity Assay Kit enables rapid measurement of 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.

Applications

Rapid assessment of PON1 activity in biological fluids (serum/plasma) or recombinant PON1 preparations.

Storage

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the Paraoxonase Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.

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

• PON1 Inhibitor (2-hydroxyquinoline): Reconstitute with 110 µl of DMSO and vortex to yield a 50 mM stock solution. To prepare a 2mM working solution (10X final concentration), add 40 µl of the 50 mM stock solution to 960 µl of dH2O. The 2 mM working solution should be stored at -20°C and is stable for 3 freeze/thaw cycles.

• PON1 Substrate: Reconstitute with 44 µl of DMSO to obtain a 250X stock solution. Store at -20°C, stable for 3 freeze/thaw cycles.

• Paraoxonase Positive Control: Reconstitute with 110 µl Paraoxonase Assay Buffer and mix thoroughly. Aliquot and store at -80°C, avoid repeated freeze/thaw cycles.

Shipping

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Gel Pack

Size

100 assays

Kit Components

Paraoxonase Assay Buffer 50 ml NM
Fluorescence Standard 1 vial Yellow
PON1 Inhibitor (2-hydroxyquinoline) 1 vial Clear
PON1 Substrate 1 vial Red
Paraoxonase Positive Control 1 vial Violet

Materials Required but Not Supplied

- Multiwell fluorescence microplate reader
- Precision multi-channel pipette and reagent reservoir
- Anhydrous (reagent grade) DMSO
- Black 96-well plates with flat bottom

Compatible Sample Types

- Human or animal plasma and serum
- Heterologously expressed recombinant PON1 preparations

Assay Protocol

1. Standard Curve Preparation: Dilute the Fluorescence Standard by adding 10 µl of the 5 mM stock to 990 µl Paraoxonase Assay Buffer to obtain a 50 pmol/µl Standard solution. 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.
2. Sample Preparation: a. Collect plasma or serum samples by standard methods (keep on ice for immediate use or aliquot and store at -80°C for future experiments). For human samples, we recommend adding 5 µl of undiluted serum/plasma per reaction, although volumes of 2-10 µl per

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reaction may be used. The sample volume and/or dilution factor required can vary based upon the nature of the sample. For unknown samples, we suggest doing a pilot experiment by testing several amounts to ensure the readings are within the range of the standard curve.
Note: As PON1 is a strongly Ca2+ -dependent enzyme, heparinized plasma samples should be used—plasma specimens collected with EDTA or other Ca2+ -chelating anticoagulants may exhibit reduced PON1 activity.
b. Prepare assay reaction wells according to the table below. In addition to the test sample wells, prepare a background control (no enzyme) well to correct for potential non-enzymatic substrate hydrolysis. For further verification of PON1 specific activity, you may prepare PON1 inhibitor control wells (sample + 200 µM 2-hydroxyquinoline) using the 2-hydroxyquinoline 2 mM working solution (10X final concentration). If desired, you may also prepare positive control (PC) and PC + inhibitor wells using the reconstituted Paraoxonase Positive Control. Adjust the volume of all reaction wells to 80 µl/well with Paraoxonase Assay Buffer:
Test Sample + PON1 Inhibitor Background Positive Control PC + Inhibitor
Sample (Serum/Plasma) 2–10 µl 2–10 µl — — —
Paraoxonase Positive Control — — — 10 µl 10 µl
2-hydroxyquinoline Solution (10X) — 10 µl — — 10 µl
Paraoxonase Assay Buffer to 80 µl to 80 µl 80 µl 70 µl 60 µl
3. Reaction Mix:
a. Preincubate the plate for 10 min 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).
b. 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. Do not add PON1 Substrate solution to the standard curve wells.
3. Measurement: Immediately (within 1 min) begin measuring the fluorescence at Ex/Em = 368/460 nm in kinetic mode for 60 min 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

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measurement time for the linear range may vary depending upon the sample. The standard curve wells may be read in endpoint mode (Ex/Em = 368/460 nm).

4. Calculations: 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. For the reaction wells (including background control), choose two time points (t1 and t2) in the linear phase of the reaction progress curves, obtain the corresponding fluorescence values at those points (RFU1 and RFU2) and determine the change in fluorescence over the time interval: ΔF = RFU2 – RFU1. Calculate specific fluorescence (CS) by subtracting the background control from each sample: CS = ΔFS – ΔFBC. Paraoxonase activity is obtained by applying the CS values to the fluorescence standard curve to get B pmol of substrate metabolized during the reaction time.

Sample Paraoxonase 1 (PON1) Activity = (B/ ΔT×V) × D = pmol/min/ml = μU/ml

Where: B is the amount of metabolite produced, calculated from the standard curve (in pmol)

ΔT is the linear phase reaction time t2 – t1 (in minutes)

V is the volume of sample added to the well (in ml)

D is the sample dilution factor (if applicable)

Paraoxonase Unit Definition: One unit of paraoxonase activity is the amount of enzyme that generates 1 μmole of fluorescent product per min at 37°C and pH 8.