Recombinant Human PON1 His-tag Fc Chimera

Catalog Number: 10175-PO

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human PON1 protein Leu16-Leu355 Accession # P27169 with an N-terminal 6-His tag and a C-terminal Fc tag
N-terminal Sequence Analysis	His
Predicted Molecular Mass	66 kDa

SPECIFICATIONS

SDS-PAGE	69-79 kDa, under reducing conditions
Activity	Measured by its ability to hydrolyze phenyl acetate. The specific activity is >5000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, $CaCl_2$, NaCl, and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 5 mM $CaCl_2$, pH 8.0
- Deionized Water
- Recombinant Human PON1 His-tag Fc Chimera (rhPON1) (Catalog 10175-PO)
- Substrate: Phenyl Acetate (7.88 M) (Sigma, Catalog # 108723)
- UV Plate (Catalog # 3635)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhPON1 to 10 µg/mL in Assay Buffer.
2. Dilute Phenyl Acetate to 40 mM in deionized water.
3. Load 50 µL of 10 µg/mL rhPON1 in a plate, and start the reaction by adding 50 µL of Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of Substrate.
4. Read plate at a wavelength of 270 nm (absorbance) in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}^{*} \text{ (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{ext. coeff}^{**} \text{ } (M^{-1}cm^{-1}) \text{ x path corr.}^{***} \text{ (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Using the extinction coefficient 1310 M-1cm-1
***Using the path correction 0.32 cm
Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

Per Well:

- rhPON1: 0.5 µg
- Phenyl Acetate: 20 mM

PREPARATION AND STORAGE

Shipping

The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage

Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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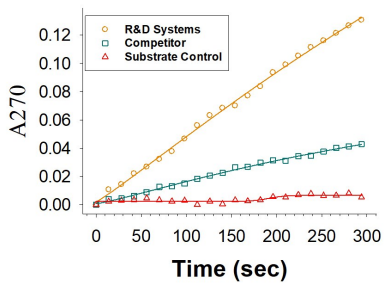
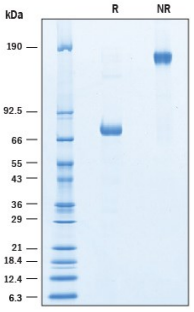
Enzyme Activity



Recombinant Human PON-1 His-tag Fc Chimera (Catalog # 10175-PO) is measured by its ability to hydrolyze phenyl acetate. The activity (orange) is over 3-fold higher than the competitor's PON-1 (green).

SDS-PAGE



2 µg/lane of Recombinant Human PON1 His-tag Fc Chimera was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® blue staining, showing a band at ~74 kDa under reducing conditions.

BACKGROUND

Serum paraoxonase 1 (PON-1) is a member of the paraoxonase family which has three members: PON1, PON2, PON3. PON-1 is a lactonase (1,2) but has significant promiscuity (2,3) that allows hydrolysis of a variety of substrates including organophosphate triesters (including many pesticides), arylesters, cyclic carbamates, glucuronides and consequently also pharmaceuticals such as statins. PON-1 is a calcium-dependent, secreted, 43 kDa protein with a 6-bladed propeller structure and an active site gorge containing a His-His catalytic dyad (4). It is a homodimer and retains its hydrophobic signal sequence in the N-terminal region which enables its association with HDL, resulting in its localization predominantly in the plasma (5,6). Low serum PON1 and dysfunctional HDL is associated with many diseases with an inflammatory component including diabetes mellitus (7,8), rheumatoid arthritis, hepatic and renal diseases, psoriasis, and macular degeneration (9,10). PON1 shows a variety of atheroprotective properties (6,11,12) by metabolizing inflammatory lipid peroxides (13). PON-1 activity is low in coronary heart disease patients (14) and contributes to an increased risk of a major adverse cardiovascular event (15). PON-1 also protects against toxicity of organophosphorus compounds used as pesticides (16,17) thought to increase risk of neurodegenerative disorders such as Parkinson's (18) and Amyotrophic Lateral Sclerosis (19).

References:

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