rpHBH

recombinant ρ-Hydroxybenzoate hydroxylase EC 1.14.13.2

from Bacteria

Reaction Equation

4-Hydroxybenzoate + NADPH + H^+ + O_2
→ 3,4-Dihydroxybenzoate + $NADP^+$ + H_2O

Specification

Specific Activity

U/mg protein	> 50 units

Contaminants

NADPH oxidase	< 0.01%
Glutathione reductase	< 0.01%
Cholinesterase	< 0.003%

Properties

pH stability	:	pH 5.5 - 7.5 (4°C, 2 week)
Thermal stability	:	≦ 30°C (pH 8.0, 15 min)
Optimum pH	:	7.0 - 7.5
Optimum temp.	:	37 - 40°C
Km value	:	2.1 × 10^{-5} mol/L (ρ-Hydroxybenzoate)
		2.5 × 10^{-4} mol/L (NADPH)
		2.0 × 10^{-7} mol/L (FAD)
Molecular weight	:	44 kDa (SDS-PAGE)

Assay Procedure

I Spectrophotometric Method

Wavelength : 340 nm, Light path length : 1 cm
Final volume : 3.02 mL, Temperature : 37°C

Pipette the following reagents into a cuvette

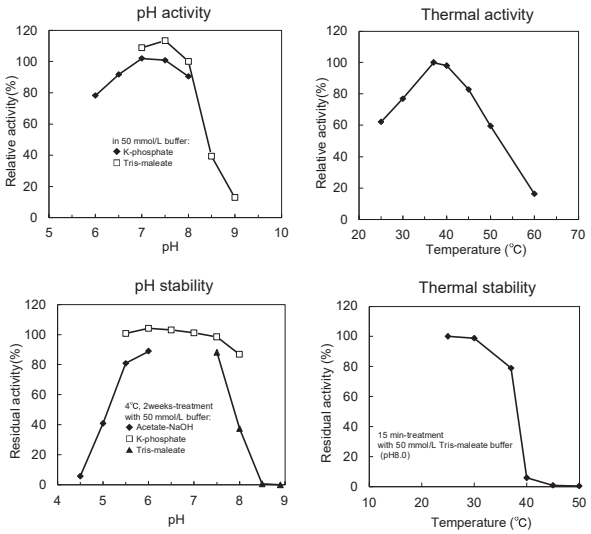
3.00 mL	Tris-maleate buffer (50 mmol/L, pH 8.0) containing ρ-Hydroxybenzoate (0.5 mmol/L) FAD (0.02 mmol/L) and NADPH (0.3 mmol/L)
0.02 mL	rpHBH (approx. 3 U/mL)

II Calculation

$$\frac{\Delta\ A/min \cdot V \cdot D}{6.2 \cdot d \cdot v} = U/mL$$

Δ A/min = The change in absorbance at 340 nm/minute
V = Total volume of reaction mixture (3.02 mL)
D = Enzyme dilution factor
6.2 = mmol/L extinction coefficient of NADPH
(L·$mmol^{-1}$·cm^{-1})
d = Light path length (1 cm)
v = Volume of enzyme sample (0.02 mL)

Reference Data



Preparation and Storage

Lyophilized powder
Store below -20°C

Cat. No./Package

Cat. No.	Package
46853903	Bulk

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