

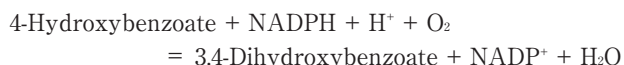
rpHBH

ρ -Hydroxybenzoate hydroxylase, recombinant from bacteria

4-Hydroxybenzoate, NADPH : oxygen oxidoreductase (3-hydroxylating) (EC 1.14.13.2)

Host cell : *E. coli*

Reaction Equation



Specification

Specific Activity

IU/mg protein

Specifications

>50 units

Contaminants

NADPH oxidase
Glutathione reductase
Cholinesterase

<0.01%

<0.05%

<0.003%

Profile

pH stability : pH 6.0 - 7.5 (4°C, 1 week)

Thermal stability : $\leq 37^\circ\text{C}$ (pH 8.0, 15 min)

Optimum pH : 7.0 - 7.5

Optimum temperature : 40 - 45°C

K_m value : 0.7 $\mu\text{mol/L}$ (FAD)

170 $\mu\text{mol/L}$ (NADPH)

70 $\mu\text{mol/L}$ (ρ -Hydroxybenzoate)

MW : 44 kD (SDS-PAGE)

Assay Procedure

I. Spectrophotometric Method

Wavelength ; 340 nm, Light path length ; 1 cm,

Temperature ; 37°C

Pipette the following reagents into a cuvette

3.00 mL Tris-maleate buffer (50 mmol/L, pH 8.0, 37°C)

containing ρ -Hydroxybenzoate

(0.5 mmol/L), FAD (0.02 mmol/L),

NADPH (0.3 mmol/L)

0.02 mL rpHBH (about 3 IU/mL)

II. Calculation

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

$\Delta A/\text{min}$ = The change in absorbance at 340 nm/minute

V = Total volume of reaction mixture (3.02 mL)

D = Enzyme dilution factor

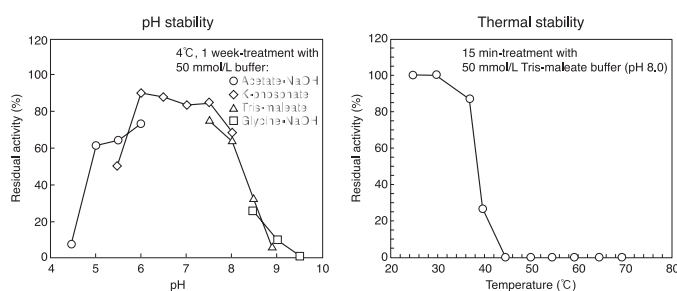
6.2 = mM extinction coefficient of NADPH

($\text{L} \cdot \text{mmol}^{-1} \cdot \text{cm}^{-1}$)

d = Light path length (1 cm)

v = Volume of enzyme sample (0.02 mL)

Reference Data



Preparation and storage

Product Code : rpHBH-03

Lyophilized powder

(contains no ammonium sulfate).....below -20°C

IU per 1 ml solution is >400 units.

OYC No./Package

OYC No.	Package
46853004	200 units
46851904	Bulk

(Research reagent use only, not for medical use.)



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