

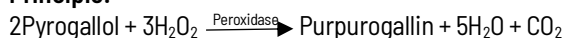
SPECIFICATION SHEET

Product Name: Peroxidase
Catalog # PER01D1-E51A

Product Description: This enzyme is a diagnostic grade reagent from a microorganism host. It is useful for enzymatic determination of H_2O_2 in clinical analysis. On the other hand, the enzyme contributes for the reduction of phenol in wastewater.

EC 1.11.1.7

Storage and Stability: Product can be stored at 2–8°C for transportation process up to ten days but long-term storage should be at –20°C.

Principle:


The appearance of purpurogallin is measured at 420 nm by spectrophotometry.

Unit Definition	: One purpurogallin unit causes the formulation of one micromole of purpurogallin in 20 seconds at 20°C and pH 6.0.	
Appearance	: Reddish brown amorphous powder, lyophilized	
Activity	: ≥ 180 U/mg solid	
Contaminants	: Phosphatase	$\leq 1.0 \times 10^{-3} \%$
Molecular Weight	: 45 kDa	
Inhibitors	: Cyanide, sulfide, fluoride, azide	
Optimum pH	: 6.5	(Figure 1)
Optimum Temperature	: 45°C	(Figure 2)
pH Stability	: pH 5.0–10.0 (25°C, 20 hr)	(Figure 3)
Thermal Stability	: Below 50°C (pH 6.0, 10 min)	(Figure 4)
Effect of various chemicals	: (Table 1)	

Table 1. Effect of Various Chemicals on Peroxidase

The enzyme dissolved in 0.1 M phosphate buffer, pH 6.0 (50 U/mL) was incubated with each chemical at 25°C for 1 hr.

Chemical	Conc. (mM)	*Residual activity (%)	Chemical	Conc. (mM)	*Residual activity (%)
None	—	100.0	MIA	2	99
CaCl_2	2	102	NaF	2	98
MgCl_2	2	102	NaN_3	2	75
ZnCl_2	2	99	o-Phenanthroline	2	98
NiCl_2	2	96	a, a'-Dipyridyl	1	96
CoCl_2	2	97	Borate	50	98
MnCl_2	2	97	Hydroxylamine	2	98
FeCl_3	2	98	EDTA	5	95
CuSO_4	2	98	Na-Cholate	0.1% (v/v)	97
AgNO_3	2	91	Tween-20	0.1% (v/v)	89
HgCl_2	2	92	Triton X-100	0.1% (w/v)	98
2-Mercaptoethanol	2	94	Brij-35	0.1% (v/v)	80
PCMB	1	98	Span-20	0.1% (v/v)	98
NEM	2	97	SDS	0.05% (w/v)	98

Table 1(Continued)

Chemical	Conc. (mM)	*Residual activity (%)	Chemical	Conc. (mM)	*Residual activity (%)
IAA	2	99	DAC	0.05% (w/v)	102

*: Residual activity was measured by 4AA-DEA method (4AA, 4-Aminoantipyrine; DEA, Diethylaniline; PCMB, p-Chloromercuribenzoate; MIA, Monoiodoacetate; DAC, Dimethylbenzylalkylammonium chloride)

Fig.1. pH-Activity

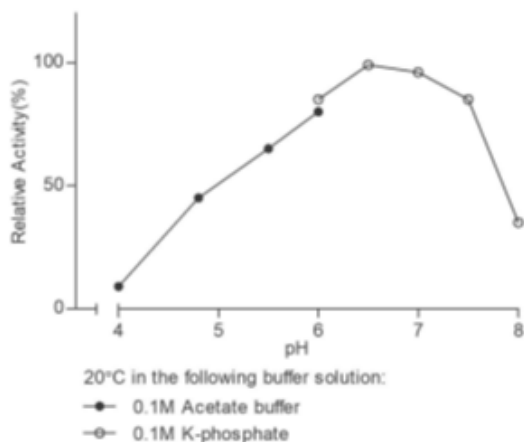


Fig.2. Temperature Activity

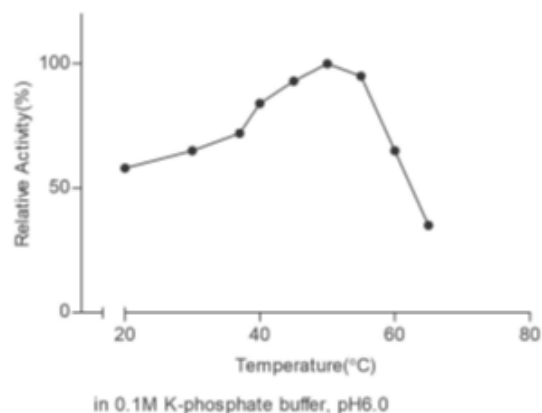


Fig.3. pH-Stability

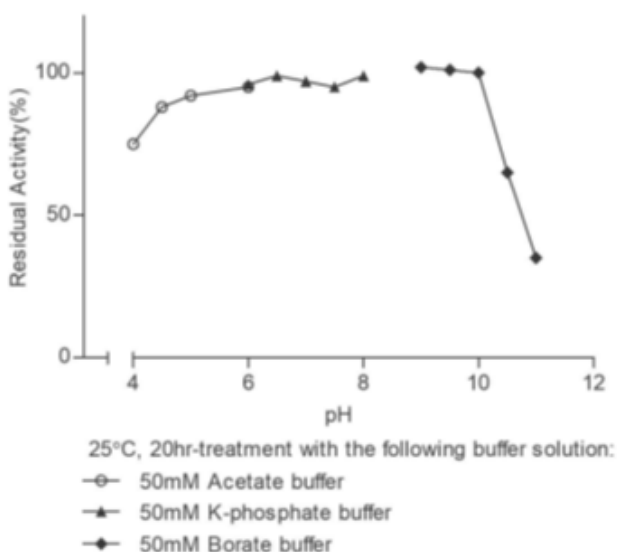
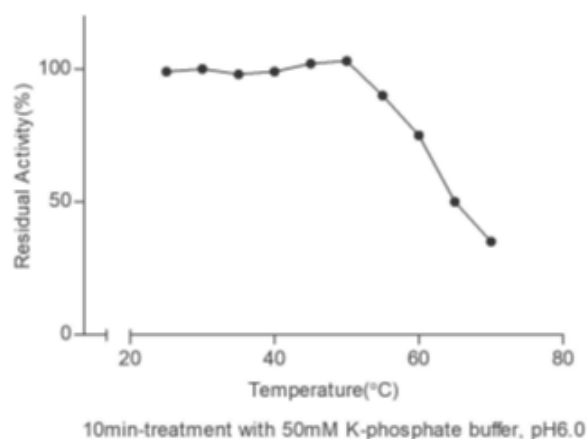


Fig.4. Thermal Stability



Assay:

Reagents:

- | | |
|--|---|
| A. Pyrogallol solution | : 5% (w/v) (Should be prepared fresh) |
| B. H ₂ O ₂ solution | : 0.147 mM [Dilute 1.67 mL of 30% (w/v) H ₂ O ₂ to 100 mL with H ₂ O] (Should be prepared fresh) |
| C. Phosphate buffer, pH 6.0 | : 100 mM |
| D. H ₂ SO ₄ solution | : 2 N |

Procedure:

1. Prepare the following reaction mixture in a test tube (32φ × 200 mm) and equilibrate at 20°C for about 5 minutes.

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14.0 mL	H ₂ O		Concentration in assay mixture	
2.0 mL	Pyrogallol solution	(A)	Phosphate buffer	15 mM
1.0 mL	H ₂ O ₂ solution	(B)	Pyrogallol	40 mM
2.0 mL	Phosphate buffer, pH 6.0	(C)	H ₂ O ₂	7.4 mM

2. Add 0.1 mL of the enzyme solution* and mix.

3. After exactly 20 seconds at 20°C, add 1.0 mL of 2.0 N H₂SO₄ solution (D) to stop the reaction.

4. Extract the produced purpurogallin from the above stopped reaction mixture in five times with 15 mL portions of ether and fill up the combined ether extracts to 100 mL with fresh ether.

5. Measure the optical density at 420 nm against water (OD test)

At the same time, prepare the blank by first mixing the reaction mixture with 1.0 mL of 2 N H₂SO₄ solution (D) after 20 a sec-incubation at 20°C, followed by the addition of the enzyme solution and extracting with ether by the same procedure as the test (ΔOD Blank).

*: Dissolve the enzyme preparation in ice-cold 0.1 M phosphate buffer, pH 6.0 (C) and dilute to 3.0~6.0 pur-purogallin U/mL with the same buffer and store on ice.

Calculation:

Activity** can be calculated by using the following formula:

$$\text{Volume Activity (U/mL)} = \frac{\Delta OD (\Delta OD_{\text{test}} - \Delta OD_{\text{blank}}) \times df}{0.117 \times V_s} = \Delta OD \times 8.547 \times df$$

Weight activity (U/mg) = (U/mL) × 1/C

V_s : Sample volume (1.0 mL)

0.117 : Optical density at 420 nm corresponding to 1 mg % of Purpurogallin in ether.

df : Dilution factor

C : Enzyme concentration in dissolution (C mg/mL)

**: One purpurogallin unit is equivalent to 13.5 international units determined with o-dianisidine at 25°C.