Peroxidase (POD) Assay Kit

Cat No. POD-M-50
Size: 50 Reactions
Store at 4°C

MOLEQULE-ON®

Components	Quantity
Extraction Solution	60 ml
Solution I	60 ml
Solution Ⅱ*	0.33 ml ×2
Solution Ⅲ	10 ml

Description

Peroxidase (EC 1.11.1.7) is an enzyme found broadly in biological systems that utilizes hydrogen peroxide in the oxidation of various substrates. The MOLEQULE-ON POD Assay Kit provides a simple and direct procedure for measuring peroxidase activity in a variety of biological samples.
The reaction is
2 phenolic donor +H_2O_2= 2 phenoxyl radical of the donor + 2 H_2O
The product has a absorption at 470nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, benchtop centrifuge, micropipette, cuvette, mortar.

Preparation:

- ***Working solution II: add 5ml Solution Ⅰ before use. This mixture can be used within one week.***

Protocol

I. Sample preparation:

1. Bacteria or cells:
Collect bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. Accordance ratio Bacteria or cell amount (10^4): Extraction reagent volume (ml) = 500~1000:1, suggested 5 million with 1ml Extraction reagent. Split bacteria and cell with ultrasonication (ice bath, 200W, work time 3s , interval 10s, repeat for 30 times). Centrifuge at 8000 rpm for 10 min at 4℃. Supernatant on the ice is used for test.
2. Tissue
Accordance ratio tissue weight (g): Extraction reagent volume (ml) = 1:5~10 (suggested 0.1g tissue with 1ml Extraction reagent). Fully grind on ice, centrifuge at 8000 g for 10 min at 4℃. Supernatant is used for test.
3. Serum (plasma) sample:
Detect sample directly.

II. Determination procedure:

1. Preheat spectrophotometer for 30min, adjust wavelength to 470nm, set zero with ddH_2O.

2. Place Solution I, Solution Ⅱ and Solution Ⅲ at 37℃ (mammal) or 25℃ (other species) for 10 min before determination.

3. Add reagents with the following list.

	(μL)
Sample	**15**
Distilled water	**270**
Solution I	**520**
Solution II	**130**
Solution III	**135**

4. Add the three solution into 1ml cuvette in order. Mix well immediately and then record the time. Measure the 570 nm absorbance at 30s (A1) and 1min30s (A2), ΔA＝A2-A1.

Calculations

Serum (plasma) sample

Unit definition: one unit of peroxidase activity is defined as producing a 0.001 absorbance change at 470 nm per minute in 1ml reaction volume for 1ml serum(plasma).

POD(U/mL)=ΔA×Vrv÷Vsv÷0.01÷T =7133×ΔA

Tissue, bacteria or culture cells

Protein concentration

Unit definition: one unit of peroxidase activity is defined as producing a 0.001 absorbance change at 470 nm per minute in 1ml reaction volume for per milligram of tissue protein.

POD(U/mg prot)＝ΔA×Vrv÷(Vsv×Cpr)÷0.01÷T =7133×ΔA÷Cpr

Sample weight

Unit definition: one unit of peroxidase activity is defined as producing a 0.001 absorbance change at 470 nm per minute in 1ml reaction volume for per gram of tissue.

POD(U/g fresh weight)＝ΔA×Vrv÷(W× Vsv÷Vs)÷0.01÷T =7133×ΔA÷W

Cell amount

Unit definition: one unit of peroxidase activity is defined as producing a 0.001 absorbance change at 470 nm per minute in 1ml reaction volume for per10 thousand bacteria or cells.

POD(U/10^4 cell)＝ΔA×Vrv÷(500×Vsv÷Vs)÷0.01÷T =14.27×ΔA

Vrv: Total reaction volume, 1.07mL;
Vsv: Total supernatant volume, 0.015mL;
Vs: Extraction Solution volume, 1 mL;
T: Reaction time, 1 min;
Cpr: Sample protein concentration, mg/mL;
W: Sample weight
500: Total number of bacteria or cells, 5 million.

Note:

If there are too much samples need test in one time, mix Solution I, Solution Ⅱ, Solution Ⅲ and ddH2O in proportion, pre-mixed solution can place at 37℃ (mammal) or 25℃(other species) for more than 10 min. Mix15μL sample with the 1055μL pre-mixed solution for text.

If ΔA is below 0.005, extend measure time to 5min. If ΔA exceed 0.5, dilute sample with extraction solution, multiply the corresponding dilution factor by the calculation formula.