Product Data Sheet

Product Name: Peroxidase
Cat. No.: GC61846

Chemical Properties

Cas. No.	9003-99-0		
SMILES	[Peroxidase]		
Formula		M.Wt	
Solubility	0.1 M phosphate buffer: soluble 10 mg/mL, clear, orange to red (pH 6.0)	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure			

Peroxidase

Protocol

Protocol for the Determination of Residual Hydrogen Peroxide in Milk Using Peroxidase [1]:
1. Peroxidase was dissolved in distilled water at a concentration of 1mg/mL.
2. Adjust the milk sample to room temperature. Take 10mL of the milk sample (or its dilution) and adjust the pH to 4.5 using 0.1N HCl. If the sample is diluted, use 0.01N HCl for pH adjustment.

Caution: Product has not been fully validated for medical applications. For research use only.
Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

3. Add 2mL of 0.01M acetate buffer (pH 4.5) to the sample. Dilute the mixture to 20mL with distilled water.
4. Filter the sample through filter paper to remove curd. Alternatively, centrifuge the sample at 3400×g for 10min to remove curd.
5. Prepare a blank for each sample's filtrate: take 5mL of the filtrate, add 1mL of distilled water, and 0.1mL of 1% o-dianisidine solution.
6. In another tube, add 5mL of the filtrate, 1mL of peroxidase solution, and 0.1mL of 1% o-dianisidine solution in methanol.
7. Incubate the reaction tubes at room temperature (23-26°C) for 10min.
8. Add 0.2mL of 4N HCl to each tube to terminate the reaction and stabilize the color.
9. After an additional 5min of incubation, measure the optical density of each tube at a wavelength of 400nm.
10. Prepare a standard curve using known concentrations of hydrogen peroxide standard solutions (1, 2, 3, 4, 5μg/mL) and compare the optical density to the hydrogen peroxide concentration.

Protocol for the Determination of Invertase (SUC) Activity Using Peroxidase [2]:
1. Prepare a 30mM sucrose ultrapure solution in PBS, a working solution of 100μM Amplex® Red reagent, 0.2U/ml peroxidase, and 2U/ml glucose oxidase, as per kit instructions. This solution should be prepared just before use and protected from light.
2. Pre-incubate the cells at 37°C for 60min in PBS (pH 6.5) in both the AP (apical) and BL (basolateral) compartments to remove glucose that can interfere with the reaction. After pre-incubation, wash the cells twice with PBS.
3. At the end of the pre-incubation, add 1mL of the reaction buffer (30mM sucrose in PBS, pH 6.5) to the AP compartment.Add 1mL of PBS (pH 7.0) to the BL compartment. Controls can be run using 30mM mannitol instead of sucrose.
4. Collect 50μl from the AP compartment at various time intervals and transfer to a 96-well plate with a black flat bottom. Since the enzymatic activity varies during cell differentiation, it may be necessary to perform preliminary assays using different time points to fit within the linearity of the standard curve.
5. Add 50μl of the working solution to react with the 50μl samples and standards in the 96-well plate and allow to react at room temperature for 30min, protected from light. Read fluorescence in the TECAN microplate reader adjusted for excitation at 530nm and emission at 590nm, and convert to concentration after blank subtraction with reference to the standard curve.
This protocol only provides a guideline, and should be modified according to your specific needs.

Caution: Product has not been fully validated for medical applications. For research use only.
Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

Reference:
[1] Gilliland S E, Enzymatic Determination of Residual Hydrogen Peroxide in Milk1, 2, Journal of Dairy Science[J]. 1969, 52: 321-324.
[2] Ferruzza S, Rossi C, Scarino M L, et al., A protocol for in situ enzyme assays to assess the differentiation of human intestinal Caco-2 cells, Toxicology in Vitro[J]. 2012, 26: 1247-1251.

Background

Peroxidase is a widely distributed oxidoreductase in living organisms, typically utilizing hydrogen peroxide (H_2O_2) as an oxidizing agent. It catalyzes the oxidation of various aromatic compounds and is therefore broadly applied in medical diagnostics, food processing, and the chemical industry[1,2].

Reference:
[1] de Oliveira F K, Santos L O, Buffon J G, Mechanism of action, sources, and application of peroxidases, Food Res Int[J]. 2021, 143: 110266.
[2] O'Brien P J, Peroxidases, Chem Biol Interact[J]. 2000, 129: 113-139.

Caution: Product has not been fully validated for medical applications. For research use only.
Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA