Lactoperoxidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1295

(Version 1.1A)

Detection and Quantification of Lactoperoxidase (LPO) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Lactoperoxidase (EC 1.11.1.7, LPO) is a hemin containing enzyme. The enzyme also catalyzes the oxidation of phenols and aromatics in the presence of hydrogen peroxide. Bovine milk is usually used as a source for isolation and purification of LPO. However, the enzyme is also present in the milk of other species and in the secretions of other mammalian glands such as the salivary gland. LPO from bovine milk has a molecular weight of 77,500. It is a glycoprotein and may exist in two isoenzyme forms.

Lactoperoxidase Activity Colorimetric Microplate Assay Kit is a sensitive assay for determining lactoperoxidase activity in various samples. The assay is based on oxidation of ABTS. The intensity of the product color, measured at 420 nm, is proportional to the lactoperoxidase activity in the sample.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	10 ml x 1	4 °C
Substrate	10 ml x 1	4 °C, keep in dark
Dye Reagent	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
Technical Manual	1 Manual	

Note:

Dye Reagent: add 1 ml distilled water before use, vortexed; store at 4 °C for a month after reconstitution.

Positive Control: add 1 ml Reaction Buffer to dissolve before use; store at -80 °C for a month after reconstitution.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 420 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay Buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples

Detect directly, or dilute with Assay Buffer.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Control	Positive Control
Reaction Buffer	90 µl	90 µl	90 µl
Sample	10 µl	--	--
Distilled water	--	10 µl	--
Positive Control	--	--	10 µl
Dye Reagent	10 µl	10 µl	10 µl
Substrate	90 µl	90 µl	90 µl
Mix, measured at 420 nm immediately and record the absorbance of the first 10th second and 70th second.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of lactoperoxidase activity is defined as the enzyme oxidizes 1 µmol ABTS per minute at pH 6.0 and 25°C.

1. According to the protein concentration of sample

$$LPO\ (U/mg) = [(OD_{Sample(70S)} - OD_{Sample(10S)}) - (OD_{Control\ (70S)} - OD_{Control\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times C_{Protein}) / T$$

$$= 771.6 \times [(OD_{Sample(70S)} - OD_{Sample(10S)}) - (OD_{Control\ (70S)} - OD_{Control\ (10S)})] / C_{Protein}$$

2. According to the weight of sample

$$LPO\ (U/g) = [(OD_{Sample(70S)} - OD_{Sample(10S)}) - (OD_{Control\ (70S)} - OD_{Control\ (10S)})] / (\varepsilon \times d) \times V_{Total} / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 771.6 \times [(OD_{Sample(70S)} - OD_{Sample(10S)}) - (OD_{Control\ (70S)} - OD_{Control\ (10S)})] / W$$

ε: molar extinction coefficient, 43.2 L/mol/cm = 0.0432 ml/µmol/cm

d: the optical path of 96-Well microplate, 0.6 cm

$C_{Protein}$: the protein concentration, mg/ml

W: the weight of sample, g

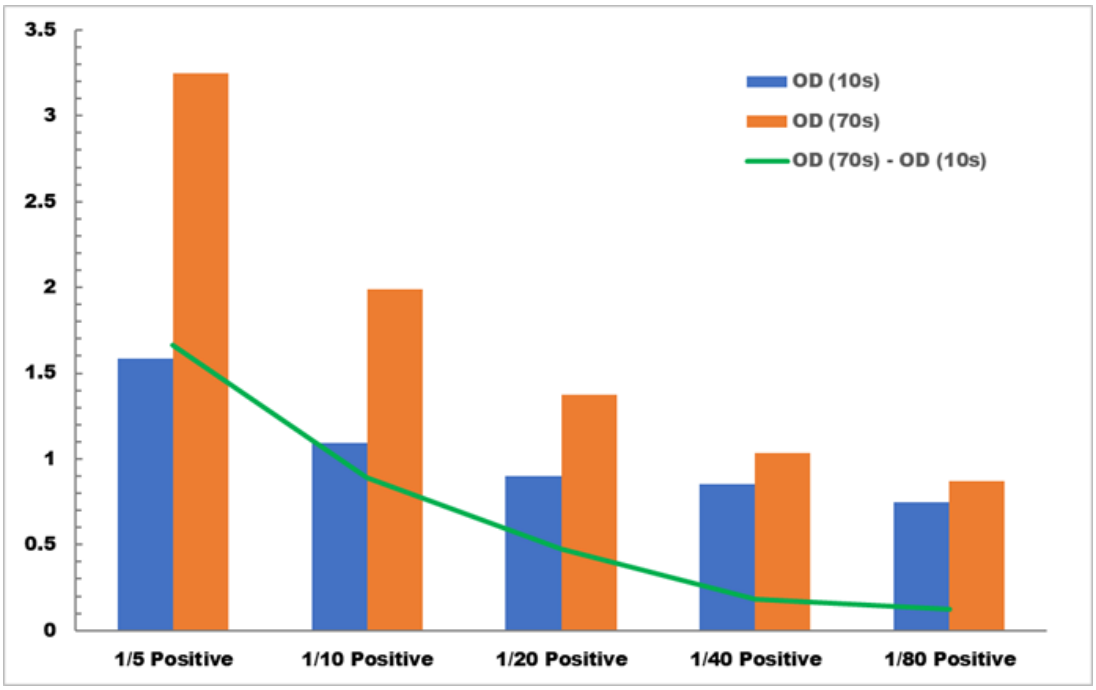
V_{Total}: the total volume of the enzymatic reaction, 0.2 ml

V_{Sample}: the volume of sample, 0.01 ml

V_{Assay}: the volume of Assay Buffer, 1 ml

T: the reaction time, 1 minutes

VII. TYPICAL DATA



Positive Control reaction in 96-well plate assay with decreasing the concentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES