Lactoperoxidase Activity

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1295

(Version 1.2A)

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

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

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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 Kitis a sensitive assay for determining lactoperoxidaseactivity 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 mlx 1	4 °C
Substrate	10 mlx 1	4 °C, keep in dark
Dye Reagent	Powder x 1	4 °C
Positive Control	Powder x 1	-20 °C
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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. REAGENT PREPARATION

Dye Reagent:Briefly centrifuge prior to opening.Add 1 ml distilled water before use, vortexed.Keep in dark for immediate use or store at 4°C for 1 day after reconstitution.

Positive Control:Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use; store at -80 °C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay 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 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer on ice, centrifuged at 8000g 4°C for 10minutes, 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.

VI. ASSAY PROCEDURE

Add following reagents intothe 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 recordthe absorbance of the first 10th second and 70th second.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

Unit Definition: One unit of lactoperoxidaseactivity is defined as the enzymeoxidizes 1 µmol ABTS per minute at pH 6.0 and 25°C.

1.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(OD_{Sample(70)} - OD_{Sample(10)}) - (OD_{Control(70)} - OD_{Control(10)})}{\varepsilon \times d \times C_{Protein} \times T} \times \frac{V_{Total}}{V_{Sample}} (U/mg)$$

1.2 According to the weight of sample

$$\text{Activity} = \frac{(OD_{Sample(70)} - OD_{Sample(10)}) - (OD_{Control(70)} - OD_{Control(10)})}{\varepsilon \times d \times W \times T} \times \frac{V_{Total} \times V_{Assay}}{V_{Sample}} (U/g)$$

1.4 According to the volume of sample

$$\text{Activity} = \frac{(OD_{Sample(70)} - OD_{Sample(10)}) - (OD_{Control(70)} - OD_{Control(10)})}{\varepsilon \times d \times T} \times \frac{V_{Total}}{V_{Sample}} (U/mg)$$

ε: molar extinction coefficient,43.2L/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

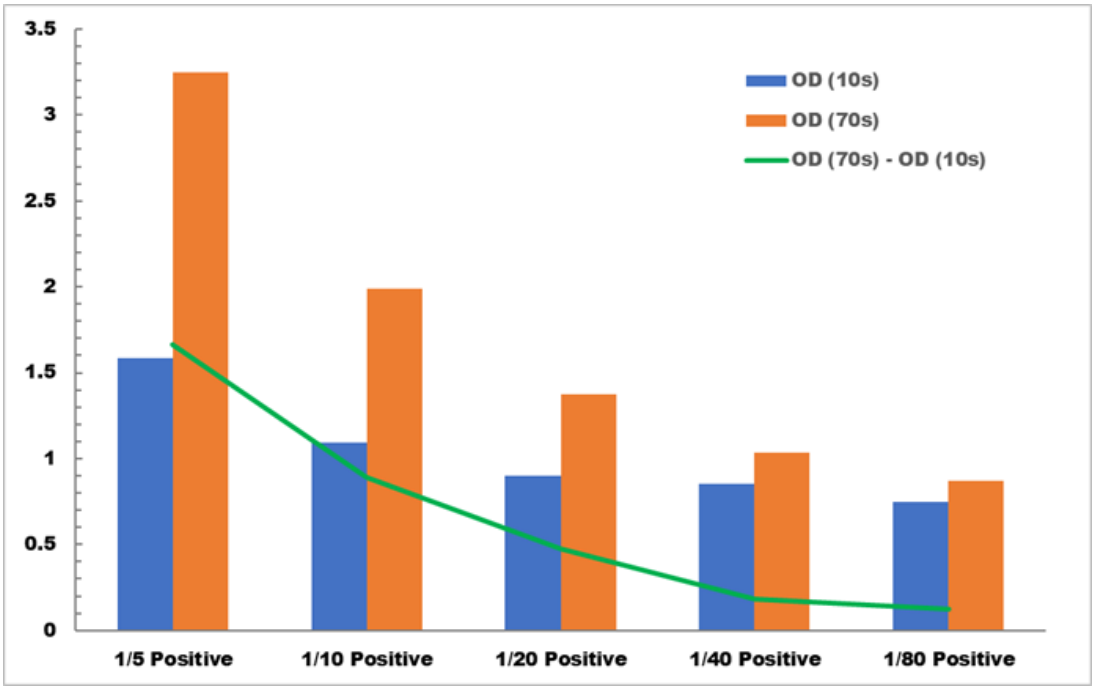
V_{Total}: thetotal volume of all reagents in assay procedure, ml

V_{Sample}: the volume of sample in assay procedure, ml

V_{Assay}: the volume of Assay Buffer II in sample preparation, ml

T: the reaction time, minutes

VIII. TYPICAL DATA



Positive Control reaction in 96-well plate assay with decreasing theconcentration