Hydroxyl Radical Microplate Assay Kit

User Manual

Catalog # CAK1285

(Version 1.1A)

Detection and Quantification of Hydroxyl Radical Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Hydroxyl radicals are a type of free radical with the most active chemical properties. They have a high reaction rate constant and cause the most harm among the free radicals. In a biological body, hydroxyl radicals attack the cell membrane, causing membrane damage and destroying sugar groups and DNA base sequences, inducing the disintegration of the double-helix structure, even causing cell death and mutations. Therefore, the scavenging activity of the hydroxyl radical is commonly used to evaluate the ability of scavenge free radicals of substance.

Hydroxyl Radical Microplate Assay Kit provides a simple and direct procedure for measuring hydroxyl radical content in a variety of samples. In this assay, H_2O_2 and Fe^{2+} generates hydroxyl radicals through the Fenton reaction, and salicylic acid can effectively capture the generated hydroxyl radicals and react with them to form colored substances with a maximum absorption at 510 nm. The ability of the sample to scavenge hydroxyl radicals is judged according to the absorbance reduce.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Reaction Buffer	Powder x 1	4 °C
Dye Reagent	9 ml x 1	4 °C
Substrate	1 ml x 1	4 °C, keep in dark
Standard (20 mmoI/L)	1 ml x 1	4 °C, keep in dark
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Note:

Reaction Buffer: add 9 ml distilled water to dissolve before use, mix.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 510 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. 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 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer, centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

If the samples may produce the hydroxyl radical, please follow this protocol.

Reagent	Standard	Blank	Sample
Reaction Buffer	90 µl	90 µl	90 µl
Standard	10 µl	--	--
Distilled water	10 µl	20 µl	10 µl
Sample	--	--	10 µl
Dye Reagent	90 µl	90 µl	90 µl
Mix, measured at 510 nm and record the absorbance.			

If the samples may inhibit the hydroxyl radical, please follow this protocol.

Reagent	Standard	Blank	Sample	Control
Reaction Buffer	90 µl	90 µl	90 µl	90 µl
Standard	10 µl	--	--	--
Distilled water	10 µl	20 µl	--	10 µl
Substrate	--	--	10 µl	10 µl
Sample	--	--	10 µl	--
Dye Reagent	90 µl	90 µl	90 µl	90 µl
Mix, measured at 510 nm and record the absorbance.				

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 activity is lower, please add more sample into the reaction system; or increase the reaction time; if the 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

※If the samples may produce the hydroxyl radical:

1. According to the volume of sample

• $OH\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$

$= 20 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

2. According to the weight of sample

• $OH\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$

$= 20 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the quantity of cell or bacteria

• $OH\ (\mu mol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$

$= 20 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$

※If the samples may inhibit the hydroxyl radical:

1. According to the volume of sample

• $OH\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$

$= 20 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank})$

2. According to the weight of sample

• $OH\ (\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$

$= 20 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / W$

3. According to the quantity of cell or bacteria

• $OH\ (\mu mol/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$

$= 20 \times (OD_{Control} - OD_{Sample}) / (OD_{Standard} - OD_{Blank}) / N$

$C_{Standard}$: the standard concentration, 20 mmol/L = 20 μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N × 10^4;

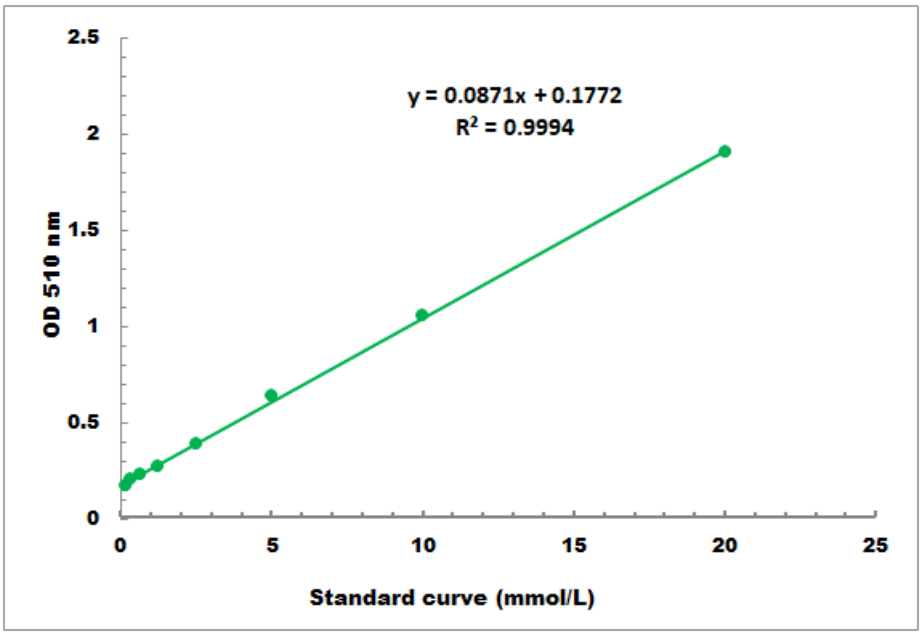
V_{Sample}: the volume of sample, 0.01 ml;

$V_{Standard}$: the volume of standard, 0.01 ml;

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

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.2 mmol/L - 20 mmol/L

VIII. TECHNICAL SUPPORT

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

IX. NOTES