# Hydroxyl RadicalMicroplate Assay Kit

# User Manual

**Catalog # CAK1285**

(Version 1.1A)

Detection and Quantification of Hydroxyl RadicalContent inUrine,Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

**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 radicalcontent 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 tothe 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 (20mmoI/L) | 1 ml x 1 | 4 °C, keep in dark |
| Technical Manual | 1 Manual | |

**Note:**

**Reaction Buffer**:add 9 ml distilled waterto 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 mlAssay buffer for 5 $\times 10^6$ 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 mlAssay 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 reagentsto 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 recordthe 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 recordthe 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 (μmol/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 (μmol/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 (μmol/$10^4$) = $(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay})$

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

※If the samples may inhibit the hydroxyl radical:

1. According to the volume of sample

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

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

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

W: the weight of sample, g;

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

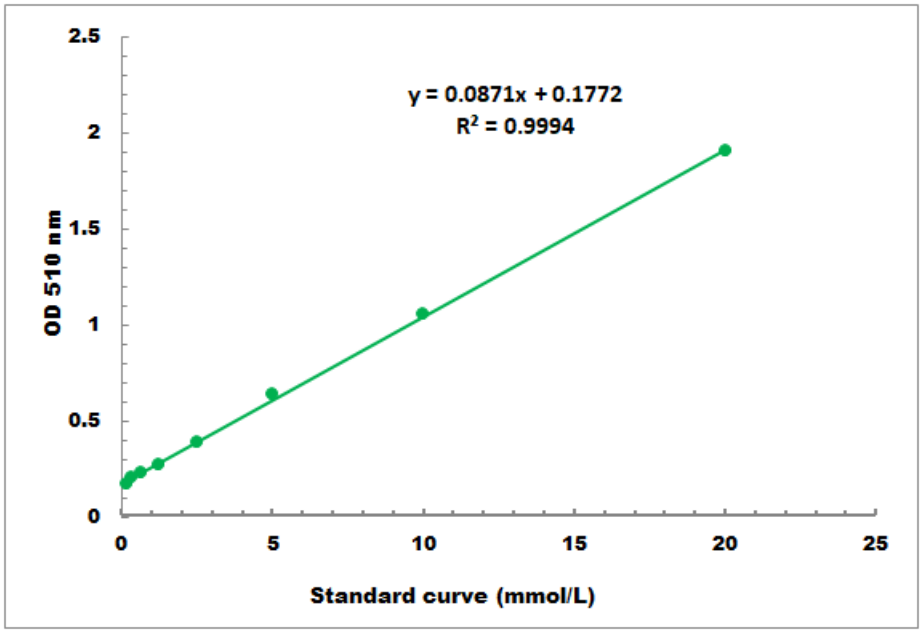
$V_{Sample}$: the volume of sample, 0.01 ml;

$V_{Standard}$: 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 towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

## IX. NOTES