Hydroxyl Radical Assay Kit

ARG83555 Hydroxyl Radical Assay Kit can be used to measure Hydroxyl Radical in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, other biological fluids

Catalog number: ARG83555

Package: 100 tests

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PRINCIPLE OF THE ASSAY

ARG83555 Hydroxyl Radical 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.

MATERIALS PROVIDED & STORAGE INFORMATION

Store all component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard (20 mmol/L)	1 ml	4 °C
Reaction Buffer	1 vial (lyophilized)	4 °C
Dye Reagent	9 ml	4 °C
Substrate	1 ml	4 °C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 510nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store all component at 2-8°C. Use the kit before expiration date.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and Bacteria-Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation. Mix and sonicate with 1 ml Assay buffer per 5×10^6 cell or bacteria. Centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue- 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.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Reaction Buffer:** Reconstitute the **Reaction Buffer** with **9 ml** of distilled water. Allow the **Reaction Buffer** keep on bench for few minutes. Make sure the **Reaction Buffer** is dissolved completely and mixed thoroughly before use.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

Summary of Hydroxyl Radical Assay Kit Procedure

Reagent	Sample	Standard	Blank
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 well. Read the OD at 510nm			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

2. Calculation:

Formula:

a). According to the volume

OH (µmol/ml) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}]$

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

b). According to the weight

OH (μmol/g) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times W \times V_{Sample} \times V_{Assay}]$

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

C). According to the quantity of cell or bacteria

OH ($\mu mol/10^4$) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / [(OD_{Standard} - OD_{Blank}) \times N \times V_{Sample} \times V_{Assay}]$

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