Chloral Assay Kit

ARG83424 Chloral Assay Kit can be used to measure Chloral in water and other biological fluids.

Catalog number: ARG83424

Package: 96 wells

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INTRODUCTION

Chloral, also known as trichloroacetaldehyde or trichloroethanal, is the organic compound with the formula Cl3CCHO. This aldehyde is a colourless liquid that is soluble in a wide range of solvents. It reacts with water to form chloral hydrate, a once widely used sedative and hypnotic substance.

PRINCIPLE OF THE ASSAY

This Chloral Assay Kit is a simple colorimetric assay that measures the amount of Chloral present in water and other biological fluids. The increase in absorbance at 480 nm is directly proportional to the Chloral.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
96 Well microplate	1 plate	
Standards	1 vial (lyophilized)	4°C
Reaction Buffer	5 mL	4°C
Reagent Dye	1 vial (lyophilized)	4°C
Reagent Dye Diluent	5 mL	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 480 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in 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.

Note: For liquid sample, it can be assayed directly.

REAGENT PREPARATION

- **Reagent Dye:** Reconstitute the Reagent Dye with **5 ml** of **Reagent Dye Diluent**. Allow the Reagent Dye keep on bench for few minutes. Make sure the Reagent Dye is dissolved completely and mixed thoroughly before use.
- **Standards:** Reconstitute the Standards with **1 ml** of **Distilled water**. Allow the Standards keep on bench for few minutes. Make sure the Standards is dissolved completely and mixed thoroughly before use. Then add **50 µl standard solution** into **950 µl Distilled water**, the concentration will be **1 µmol/mL**.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **100 µl** of **Sample** into Sample wells.
2. Add **100 µl** of **Standard** into Standard wells.
3. Add **50 µl** of **Reaction Buffer** into all wells.
4. Add **50 µl** of **Reagent Dye** into all wells.
5. Mix well. Incubate at **90°C** for **15 min**.
6. Read the OD at **480 nm**.

Summary of Chloral Assay Procedure

Reagent	Sample	Standard	Blank
Sample	100 µl	-	-
Standard	-	100 µl	-
Distilled water	-	-	100 µl
Reaction Buffer	50 µl	50 µl	50 µl
Reagent Dye	50 µl	50 µl	50 µl
Mix well. Incubate at 90°C for 15 min Read the OD at 480 nm.			

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Control, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Calculation:

   A. Definition:

   $C_{Standard}$: the standard concentration, 1 µmol /ml;

   V_{Sample}: the volume of reaction sample, 100 µl = 0.1 ml;

   $V_{standard}$: the volume of standard, 100 µl = 0.1 ml;

   B. Formula:

   a). According to the volume of sample

   Chloral (µmol/ml) =

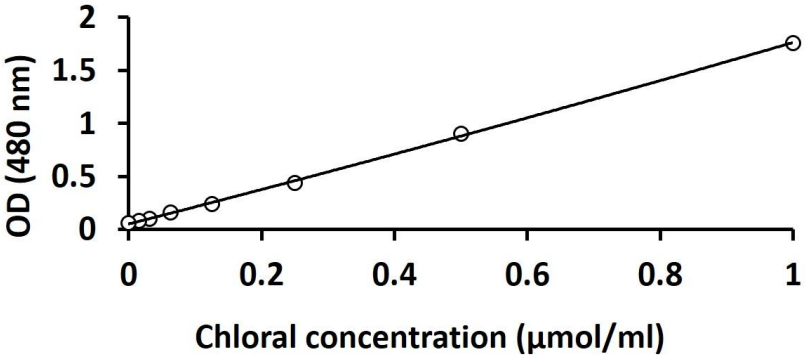
   $[(C_{Standard} \times V_{standard}) \times (OD_{Sample} - OD_{blank})] / [(OD_{Standard} - OD_{Blank}) \times V_{Sample}]$

   $= (OD_{Sample} - OD_{blank}) / (OD_{Standard} - OD_{Blank})$
5. Detection range:

   The detection range is from 0.01 µmol/ml- 1 µmol/ml.
6. If the samples have been diluted, the calculated activity must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Chloral Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.01 µmol/mL