Chloral Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6838

Detection and Quantification of Chlo ralContent in Water and Other biological fluids Samples.

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

1. Introduction

Chloral, also known as trichloroacetaldehyde or trichloroethanal, is the organic compound with the formula Cl_3CCHO. This aldehyde is a colourless oily 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.

ChloralColorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of Chloralconcentration in a variety of samples. The intensity of the product color, measured at 480 nm, is proportional to the Chloral concentration in the sample.

2. Kit Components

Component	Volume	Storage
96-Well Microplate	1 plate	-
Reaction Buffer	5 mL x 1	4 °C
Dye Reagent	Powderx 1	4 °C, keep in dark
Dye Reagent Diluent	5 mL x 1	4 °C, keep in dark
Standard	Powderx 3	4 °C, keep in dark
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Microplate reader to read absorbance at 480 nm
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Centrifuge
6. Timer
7. Hot air circulation oven

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve each vial in 1 mL distilled water to generate 20 mmol/ L of standard solution ,keep in dark for immediateuse of store at -20°C for 3 days after rec onstitution. Then dilute to 1 mmol/ Lstandard top solution by adding 50 µl stock solution into 950 µl distilled water.Perform 2 -fold serial dilutions of the top standard solution using distilled water to make the standard curve.The concentration of standard curve could be 1/0.5/0.25/0.125/ 0.0625 /0.0312 /0.0156/0.0078mmol/L.

Dye Reagent: Add 5 mL Dye Reagent Diluent, mix before use. keep in dark and store at -20°C for1month after rec onstitution.

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

5. Sample Preparation

1. For liquid samples Detect directly , or dilute with distilled water.

6. Assay Procedure

Add following reagents in to the microplate :

Reagent*	Sample**	Standard	Blank
Sample	100µl	--	--
Standard	--	100µl	--
Distilled water	--	--	100µl
Reaction Buffer	50 µl	50 µl	50 µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, cover the plate adhesive strip, put it into the hot air circulation oven, incubate at90°C for 15minutes, recordabsorbance measured at 480 nm.			

Note:

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

**The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (mmol/L)$$

Calculate the initial concentration according to sample preparation procedure .

2. According to one point of the standard OD and concentration

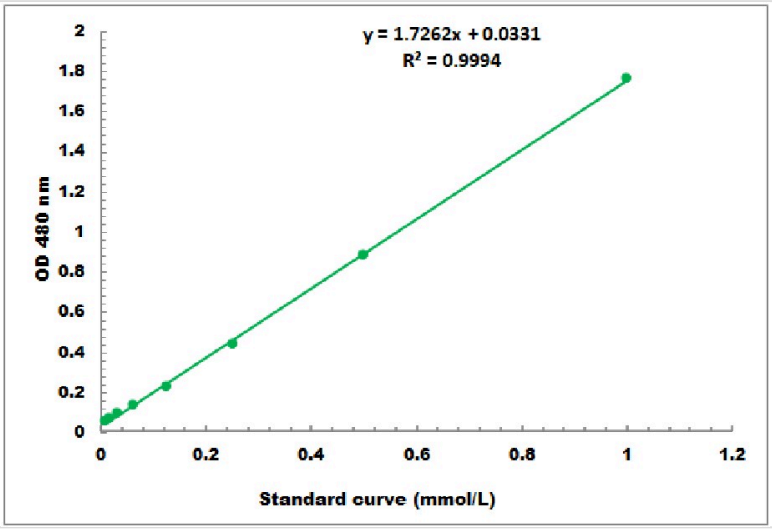
According to the volume of sample

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

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, mmol/L = μmol/ml
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl

8. Typical Data

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



Detection Range : 0.01 mmol/L - 1 mmol/L