Bilirubin

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1163

(Version 1.4C)

Detection and Quantification of BilirubinContentin Serum,Urine and Other biological fluidsSamples.

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

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I. INTRODUCTION

Bilirubin is a yellow compound that occurs in the normal catabolic pathway that breaks down heme in vertebrates. This catabolism is a necessary process in the body's clearance of waste products that arise from the destruction of aged red blood cells. Bilirubin is one of the degradation products of hemoglobin formed when red blood cells die. Bilirubin exists in the insoluble unconjugated form (also indirect bilirubin), or soluble glucuronide conjugated form bilirubin (also direct bilirubin). Conjugated bilirubin moves into the bile canaliculi of the liver and then to the gall bladder. When stimulated by eating, bile (including the conjugated bilirubin) is excreted into the small intestine, where bilirubin is converted into urobilinogen. Bilirubin is a key diagnostic indicator. High levels of bilirubin result when too much hemoglobin is broken down or the removal of bilirubin does not function properly. The accumulation of bilirubin in the body causes jaundice.

BilirubinColorimetric Microplate Assay Kit is designed to measure bilirubin in various samples in 96-wellmicroplate. The vanadate oxidation method is used for testing the bilirubin. The color intensity at 630 nm is directly proportional to Bilirubinconcentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Dye Reagent	Powder x 2	4 °C, keep in dark
Dye ReagentDiluent	20 ml x 1	4 °C
Standard	Powder x 2	-20 °C, keep in dark
Standard Diluent	10 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 630 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips

IV. REAGENT PREPARATION

Standard: Briefly centrifuge prior to opening.Dissolvein1 ml Standard Diluentto generate 5 mmol/L of standardstock solution,keep in dark and store at 4 °C for 1 dayafter reconstitution.Then dilute to200µmol/Lstandard top solutionby adding20 µl stock solution into 480µl Standard Diluent**.**Perform 2-fold serial dilutions of the top standardsolution using Standard Diluentto make the standard curve.The concentration of standard curve could be200/100/50/25/12.5/6.25/3.12µmol/L.

Dye Reagent:Add 10 mlDye ReagentDiluent to dissolve each vial before use. Store at 4 °Cand use within 8 hours after reconstitution.

V. SAMPLE PREPARATION

1.For serum,urine or other biological fluidssamples

Detect directly.

VI. ASSAY PROCEDURE

Add following reagents in the microplate:

Reagent*	Sample**	Standard	Blank
Sample	50 µl	--	--
Standard	--	50 µl	--
Distilled water	--	--	50 µl
Dye Reagent	200 µl	200 µl	200 µl
Mix, incubate at37°C for 10minutes, record absorbance measured at 630 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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

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

Calculate the initial concentration according to sample preparation procedure.

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

2.1According to the volume of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

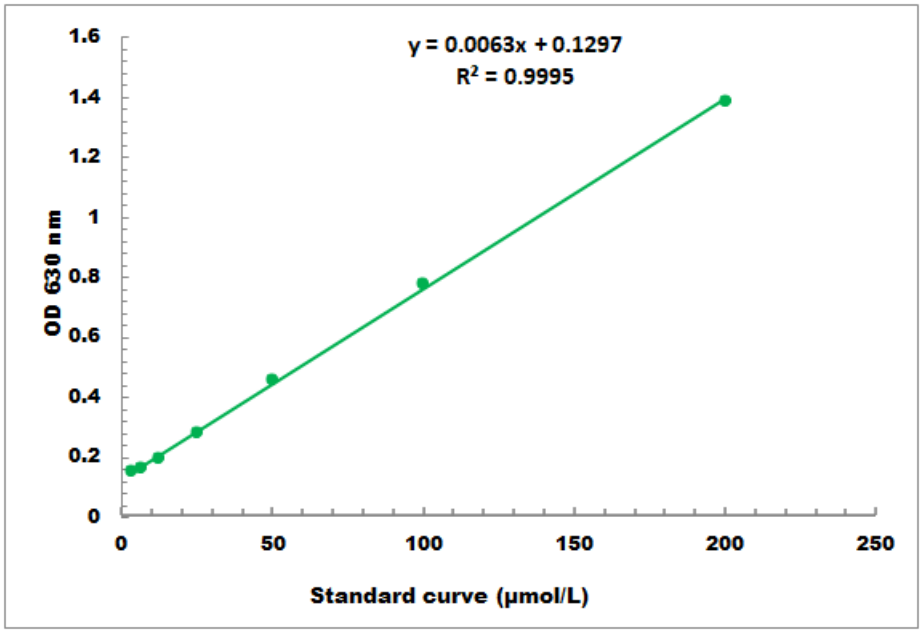
$C_{Standard}$: the standard concentration, μmol/L

$V_{Standard}$: the volume of standard in assay procedure, μl

V_{Sample}: the volume of sample in assay procedure, μl

VIII. TYPICAL DATA

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



Detection Range: 1 μmol/L -200μmol/L