Bilirubin Microplate Assay Kit

User Manual

Catalog # CAK1163

(Version 1.3B)

Detection and Quantification of Bilirubin Content in Serum, Urine and Other biological fluids Samples.

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

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.

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

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	20 ml x 1	4 °C
Standard	Powder x 2	4 °C
Standard Diluent	10 ml x 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Standard: add 1 ml Standard Diluent into the tube, vortex to dissolve; then add 40 µl into 960 µl Standard Diluent, the concentration will be 200 µmol/L.

Dye Reagent: add 20 ml Dye Reagent Diluent to dissolve before use.

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. SAMPLE PREPARATION

1. For serum, urine or other biological fluids samples

Detect directly.

V. 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 at 37 °C for 10 minutes, record absorbance measured at 630 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the volume of sample

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

$$= 200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

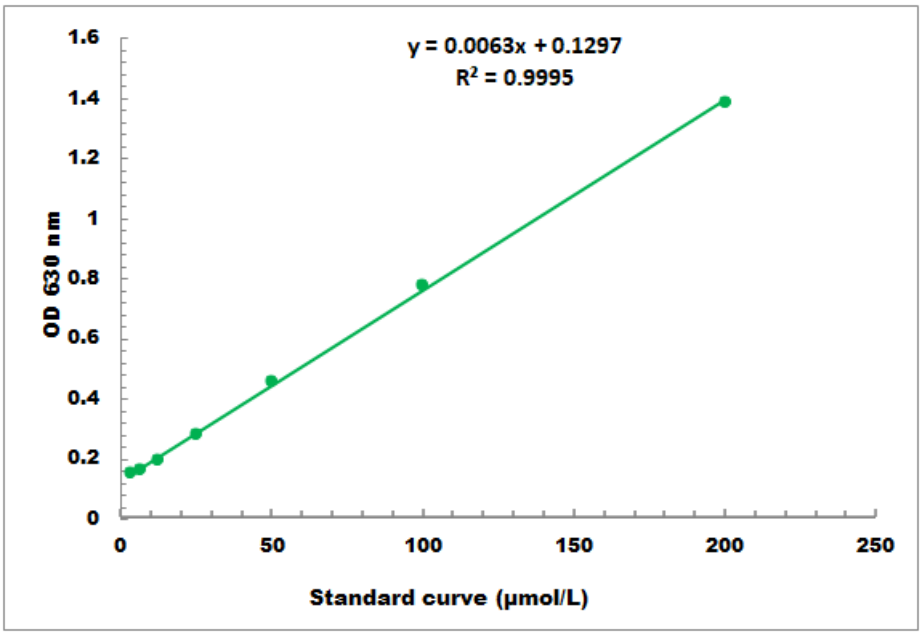
$C_{Standard}$: the standard concentration, 200 μmol/L;

$V_{Standard}$: the volume of standard, 0.05 ml;

V_{Sample}: the volume of sample, 0.05 ml.

VII. TYPICAL DATA

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



Detection Range: 1 μmol/L - 200 μmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES