CATACHEM

DIRECT BILIRUBIN REAGENT KIT
C305-0A

Contents	Product No.	Package
Direct Bilirubin Reagent Kit	**C305-0A**	
D Bilirubin Sample Diluent (R1)	C305-01	2 x 125 mL
D Bilirubin Sodium Nitrite Concentrate (R2)	C305-02	4 x 1.5 mL
D Bilirubin Sodium Nitrite Diluent	C305-03	4 x 25 mL

REAGENT PREPARATION

1. The Sample Diluent (R1) are packaged ready for use. No preparation is required.
2. Prepare the Direct Bilirubin Working Reagent (R2) by pouring the entire contents of one bottle of Sodium Nitrite Diluent into one bottle of Sodium Nitrite Concentrate. Mix well for 5-10 minutes.

REAGENT STORAGE AND STABILITY

Store unopened reagent at 15-30°C.
When stored as directed, the reagents are stable until the expiration date stated on the label.
Store opened or prepared reagents at 2-8°C.

NOT FOR USE IN UNPROFESSIONAL SETTINGS

FOR TECHNICAL ASSISTANCE:
Email: catachem@catacheminc.com
Contact Form: www.catacheminc.com
Call: 203-262-0330

353 Christian Street, Suite 2 ● Oxford, Connecticut ● 06478 USA ● Phone: (203) 262-0330 ● catachem@catacheminc.com

DIRECT BILIRUBIN REAGENT KIT
C305-0A
MANUAL/AUTOMATED APPLICATION

Intended Use
For **IN VITRO quantitative** determination of Direct Bilirubin in serum or plasma using manual or automated applications.

Clinical Significance
Measurements of Bilirubin in blood are used for diagnosing obstructive jaundice and hepatitis, as well as to monitor the causes and treatment. (1)

Method History
In 1883 Ehrlich reported the reaction of Bilirubin with diazotized sulfanilic acid to form a chromogenic complex. (2) VandenBerg applied this colorimetric reaction to the quantitative determination of Bilirubin in serum. (3) The Catachem Direct Bilirubin method for manual or automated applications is based upon the method of VandenBerg.

Method Principle
The serum sample is mixed with diazotized sulfanilic acid to form the azo-Bilirubin complex. The increase in absorbance is monitored at 550nm. The reaction scheme illustrates the reaction that occurs in this method.

$$\text{Bilirubin} + \text{diazotized-sulfanilic Acid} \xrightarrow[\text{Surfactant}]{H^+} \text{Azo -Bilirubin complex}$$

Reagent Content
The concentrations of the active ingredients in the reagent will be approximately as follows:

Direct Bilirubin Sample Diluent
Each liter contains:	
Sulfanilic Acid	14.0 mmol/L
Hydrochloric Acid	120.0 mmol/L
Nonreactive ingredients	

Sodium Nitrite Diluent
Each liter contains:	
Sulfanilic Acid	26.3 mmol/L
Hydrochloric Acid	713 mmol/L
Nonreactive Ingredients	

Sodium Nitrite
Each liter contains:	
Buffer	
Sodium Nitrite	87.5 mmol/L
Preservative	

Precautions
Avoid contact of reagent with skin and eyes. Should contact occur, wash affected area with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Preparation Of Working Reagent
Prepare the Catachem Direct Bilirubin Working Reagent by adding the contents of one vial of Sodium Nitrite to one bottle of Direct Bilirubin Nitrite Diluent. Mix well.

Reagent Storage And Stability
Catachem unopened Direct Bilirubin Reagents can be stored between 15-30˚C. Once opened or prepared, Direct Bilirubin Reagents should be stored at 2-8˚C. When stored as directed, the reagents are stable until the expiration date stated on the label. When prepared and stored as directed, the Direct Bilirubin Working Reagent is stable for 14 days at 2-8˚C. The Catachem Direct Bilirubin Reagents have been tested to reflect shipping conditions and are stable for the lifespan of the product if frozen up to 5 times or reaching temperatures up to 40°C for up to one week.

Specimen Collection And Preparation
Test sera should be fresh, clear, and non-hemolyzed. When blood is drawn, it should be processed as soon as possible and the serum should be isolated from the clot without delay.

Quality Control
To ensure optimal performance of these reagents and this procedure, we recommend systematic calibration using Catachem's Bilirubin Calibrator (C310-10). Assay performance should be monitored by running normal/abnormal controls concomitantly with samples. Catachem has optimized this assay using Catatrol Level I (C1200-11) and Catatrol Level II (C1200-12) and recommends their use for daily QC.

Interfering Substances
Various substances have been reported to interfere with the Direct Bilirubin method. (4-6) A comprehensive discussion on these interfering substances is beyond the scope of this product labeling.

Expected Values
The normal range of this assay as performed below is 0.0 mg/dL to 0.3 mg/dL. These values serve as suggested reference points only. For veterinary samples, ranges will be dependent on the species under test. It is recommended that each laboratory establish the normal ranges for the species under study and for the geographic area in which the laboratory is located.

Procedure
Important: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)
Spectrophotometer	
Matched cuvettes	1 cm light path
Timer	to time incubation time
Pipette	2.8 ml for reagent
Pipette	0.2 ml for sample

Materials Provided
Direct Bilirubin Sample Diluent, Sodium Nitrite Diluent, and Sodium Nitrite.

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Analytical Parameters

Wavelength	550nm
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	1 minute
Reagent Volume	2.8 ml
Sample Volume	0.2 ml
Total Volume	3.0 ml
Sample-to-Reagent Ratio	1:15

Note: To eliminate interferences of lipemic and other endogenous interfering substances and to maximize accuracy in the assay procedure, all samples should be blank corrected.

Assay Procedure

1. Pipette 2.8 ml of Direct Bilirubin Working Reagent into each of three cuvettes marked “Calibrator”, “Sample”, and “Blank”.
2. Pipette 0.2 ml of Calibrator or Sample into their respective cuvettes. Use 0.2 ml of water for the Blank. Mix all cuvettes well.
3. Incubate all cuvettes for exactly 1 minute at room temperature.
4. Set spectrophotometer wavelength at 550nm and zero the instrument with Blank.
5. Read the Calibrator and Sample absorbencies.
6. Calculate the Direct Bilirubin concentration (mg/dL) in the sample(s), as shown in calculations and results.

Blank Procedure

Follow the same procedure as for the assay by substituting the Working Reagent with the Direct Bilirubin Diluent.

Calculations And Results

$$\text{DBILI} = \frac{\text{Sample Absorbance}}{\text{Calibrator Absorbance}} \times \text{Calibrator (mg/dL)}$$

		Assay OD	Blank OD
Example:	Sample	0.320	0.020
	Calibrator	0.250	0.015

Calibrator = 5.0 mg/dL

$$\text{DBILI (mg/dL)} = \frac{0.320 - 0.020}{0.250 - 0.015} \times 5 \text{ mg/dL}$$

Method Performance Characteristics

= 6.4 mg/dL

***Sensitivity*:** The sensitivity of this method is 0.040 - 0.051 absorbance units per mg/dL.
***Linear Range*:** In this method there is no significant nonlinearity over the range of 0-5 mg/dL.
***Precision*:** Within-run and day-to-day precision is summarized below.

Precision Study

D Bilirubin mg/dL	Total Precision SD	Total CV %
0.47	**0.05**	*
6.46	**0.10**	**1.6**
12.53	**0.13**	**1.0**

D Bilirubin mg/dL	Within-run Precision SD	Within-run CV %
0.47	**0.04**	*
6.46	**0.08**	**1.20**
12.53	**0.05**	**0.43**

* CV% values are not meaningful when average approaches zero.

Correlation

Using a reference method based on the procedure of Van den Berg and Muller, linear regression analysis produced the following results:

Range	=	0.1 - 10.8
N	=	30
Y	=	1.000x + 0.02
r	=	0.999
Sy.x	=	0.14

References

1. Tietz N.W. (Editor) Fundamentals of Clin Chem, 2nd Ed (1982). WB Saunders Co, Philadelphia.
2. Ehrlich P. Sulfodiazobenzol ein reagens auf bilirubin. Centr Klin Medm4, 721-723 (1883).
3. Van den Bergh A.H. and Snapper J. Die farbstoffe des blutserums. Deut Arch Klin Med 110, 540-561.
4. Young D.S., Pestaner L.C., Gibberman V. Effect of drugs on clinical laboratory tests. Clin Chem 21 (5):1D-432D (1975).
5. Martin E.W. Hazards of Medication (Alexander SF, Farage D.J. and Hassan W.E. Jr. eds). Philadelphia PA and Toronto, Canada. J.B. Lippincott Co. (1971) pp 169-189.
6. Constantino N.V., Kabat H.F. Drug-induced modifications of laboratory test values. Revised 1973. Am J Hosp Pharm 30:24-71 (1973).

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