CATACHEM

TOTAL BILIRUBIN (Diazo-sulfanilic) REAGENT KIT
V314-16D, C314-0C, C314-0A

Contents	Product No.	Package
TOTAL BILIRUBIN (Diazo-sulfanilic) REAGENT KIT	**V314-16D**	
Sulfanilic Reagent (R1)	V314-17	3 x 13 mL
Nitrite Reagent (R2)	V314-18	3 x 4 mL
TOTAL BILIRUBIN (Diazo-sulfanilic) REAGENT KIT	**C314-0C**	
Sulfanilic Reagent (R1)	C314-19	2 x 25 mL
Nitrite Reagent (R2)	C314-20	2 x 7 mL
TOTAL BILIRUBIN (Diazo-sulfanilic) REAGENT KIT	**C314-0A**	
Sulfanilic Reagent (R1)	C314-01	4 x 120 mL
Nitrite Reagent (R2)	C314-02	4 x 25 mL

REAGENT PREPARATION

These reagents are packaged ready for use.
No preparation is required.

REAGENT STORAGE AND STABILITY

Store unopened reagents at 2-8°C.
When stored as directed, the reagent is stable until the expiration date stated on the label.

NOT FOR USE IN UNPROFESSIONAL SETTINGS.

FOR TECHNICAL ASSISTANCE:
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TOTAL BILIRUBIN
V314-16D, C314-0C, C314-0A
MANUAL/AUTOMATED PROCEDURE

Intended Use
For **IN VITRO quantitative** determination of Total 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.

Method History
In 1883 Ehrlich reported the reaction of Bilirubin with diazotized sulfanilic acid to form a chromagenic complex. [2] VandenBerg applied this colorimetric reaction to the quantitative determination of Bilirubin in serum. [3]. Later, Pearlman, et al [4] and Norvos, et al [5] modified the method of VandenBerg by using surfactants to free the protein bound Bilirubin without protein precipitation. The Catachem Total Bilirubin method for manual or automated applications is based upon the method of VandenBerg as modified by Pearlman [4] and Norvos [5]

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

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                                    H+
Bilirubin + Diazotized-sulfanilic Acid ------------------->
                                  Surfactant
Azo-bilirubin Complex
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Reagent Content
When reconstituted according to directions, the concentrations of the active ingredients in the reagents will be approximately as follows:

Nitrite Diluent
Each liter contains:	
Sulfanilic Acid	14.0 mmol/L
Hydrochloric Acid	0.12 mol/L
Surfactant	
Nonreactive ingredient	

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

Sample Diluent
Each liter contains:	
Sulfanilic Acid	14.0 mmol/L
Hydrochloric Acid	0.12 mol/L
Surfactant	
Nonreactive ingredient	

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 Reagents
Catachem Total Bilirubin Blank Reagent (Sample Diluent Reagent) is packaged ready-for-use. No preparation is required.

Prepare Catachem Total Bilirubin Working Reagent by adding the contents of one vial of Sodium Nitrite to one bottle of Nitrite Diluent. Using a magnetic stirrer, mix well for a minimum of 5-10 minutes.

Reagent Storage And Stability
Store Catachem Total Bilirubin Reagents 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 Total Bilirubin Working Reagent is stable for 30 days at 2-8˚C. With time, some color will appear in the Working Reagent. This does not interfere with reagent performance. Once opened the Total Bilirubin Sample Diluent is stable for 60 days at 2-8˚C. The Catachem Total Bilirubin Reagents have been tested to reflect shipping conditions and is 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 monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum as well as a calibrator. Catachem offers a bilirubin calibrator for this assay (Catalogue no. C310-10).

Interfering Substances
Various substances have been reported to interfere with the Total Bilirubin Method. [6] A comprehensive discussion on these interfering substances is beyond the scope of this product labeling.

Expected Values
The range of expected values determined for this method is 0.1 - 1.3 mg/dL. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

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

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

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TOTAL BILIRUBIN
V314-16D, C314-0C, C314-0A
MANUAL/AUTOMATED PROCEDURE

Materials Provided
Catachem Nitrite Diluent, Sodium Nitrite and Sample Diluent

Analytical Parameters

Wavelength	550nm
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	5 minutes
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
1a. Pipette 1.0 ml of Catachem Total Bilirubin Working Reagent into each of three cuvettes marked “Calibrator”, “Sample” and “Control”. (Assay cuvettes).
1b. Pipette 1.0 ml of Total Bilirubin Sample Diluent into each of three separate cuvettes marked “Calibrator”, “Sample” and “Control” (Blank cuvettes).
2. Into all cuvettes blank and assay, pipette 0.05 ml of “Calibrator”, “Sample”, and “Control” respectively.
3. Incubate all cuvettes for 5 minutes at room temperature.
4. Set spectrophotometer wavelength at 550nm and zero the instrument with blank.
5. Read the “Calibrator” “Sample” and “Control” for absorbencies for all cuvettes.
6. Calculate the Total 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 Total Bilirubin Diluent.

Calculations And Results

$$\text{T Bili} = \frac{\text{Sample (Assay OD - blank OD)}}{\text{Calibrator (Assay OD - blank OD)}} \times \text{cal. (mg/dL)}$$

	Assay OD	Blank OD
Example: Sample	0.320	0.020
Calibrator	0.250	0.015
Calibrator	= 5.0 mg/dL	

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

= 6.4 mg/dL

Method Performance Characteristics

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 0-30 mg/dL.
Precision: Within-run and day-to-day precision is summarized below.

Precision Study

T. Bili.	Within-Run		Total Precision	
Mean	SD	CV	SD	CV
mg/dL	mg/dL	%	mg/dL	%
0.45	0.09	*	0.12	*
13.15	0.11	0.86	0.16	1.20
26.50	0.16	0.61	0.39	1.5

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

Correlation
Using an automated analyzer and a reference method based on the procedure of VandenBerg and Muller resulted in the following regression statistics:

Range	=	0.3 - 16.7
N	=	108
Y	=	0.982x + 0.13
r	=	0.999
Sy.x	=	0.22

References
1. Tietz NW. (Editor) Fundamentals of Clin Chem, 2nd Ed (1982). WB Saunders Co, Philadelphia.
2. Ehrlich P. Sulfodiazobenzol ein reagens auf bilirubin. Centr Klin Med 4,721-723 (1883).
3. VandenBerg AH and Snapper J. Die farbstoffe des blutserum. Deut Arch Kiln Med 110, 540-561.
4. Pearlman FC, Lee RTY. Detection and measurement of total bilirubin in serum with use of surfactants as solubilizing agents. Clin Chem 20:447 (1974).
5. Norvos JD, Koch TR, Knoblock EC. Improved method for accurate quantitation of total and conjugated bilirubin in serum. Clin Chem 25:1891-1899 (1979).
6. Young DS, Pestaner LC, Gibberman V. Effect of drugs on clinical laboratory tests. Clin Chem 21(5):1D-432D (1975).

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