Bilirubin Direct

DMSO.Liquid

Product information

24BILD01-UN	**Meditest Bilirubin Direct**	**5x60 mL 1x10 mL**
24BILD01-AU	**Meditest Bilirubin Direct**	**5x60 mL 1x10 mL**
24BILD01-AB	**Meditest Bilirubin Direct**	**5x60 mL 1x10 mL**
24BILD01-ER	**Meditest Bilirubin Direct**	**5x60 mL 1x10 mL**
24BILD01-AR	**Meditest Bilirubin Direct**	**5x60 mL 1x10 mL**

Purpose

In vitro assay for the quantitative determination of direct bilirubin in human serum and plasma.

Summary

Bilirubin is a hemoglobin degradation product that is insoluble in water. It is transported from the spleen to the liver and excreted in the bile. Hyperbilirubinemia is caused by increased concentrations of bilirubin in plasma.
Causes of hyperbilirubinemia:
Total bilirubin: Increased hemolysis, genetic errors, neonatal jaundice, ineffective erythropoiesis and drugs.
Direct bilirubin: Hepatic cholestasis, genetic errors, hepatocellular damage[1,6,7]
Clinical diagnosis should not be based on a single test result, but clinical and other laboratory data should be integrated.

Test principle

Bilirubin is converted to colored azobilirubin by diazotized sulfanilic acid and measured photometrically. Of the two fractions present in serum, bilirubin-glucuromide and free bilirubin loosely bound to albumin, only the first reacts directly in aqueous solution (bilirubin direct), while free bilirubin must be dissolved with dimethylsulfoxide (DMSO) to react (bilirubin indirectly). In the determination of indirect bilirubin, direct is also determined, the results correspond to total bilirubin. The intensity of the resulting color is proportional to the bilirubin concentration in the sample[1,2,3]

Reagents - working solutions

R 1 Buffer	Sulfanilic acid	>30 mmol/L
	Hydrochloric acid (HCl)	>50 mmol/L
	Dimethylsulphoxide (DMSO)	>7 mol/L
R 2 Substrate	Sodium nitrite	<20 mmol/L

Precautions warnings

It is intended for in vitro diagnostic use by healthcare professionals. Follow the normal precautions necessary in handling all laboratory reagents.
Infectious or microbial waste:
Warning: handle waste as potentially biohazardous. Dispose of waste according to accepted laboratory instructions and procedures.
Environmental hazards: Follow all relevant local disposal regulations to determine that it has been disposed of safely. If requested, a safety data sheet can be provided to professional users.
Inhibit foam formation in all reagents and sample types (sample, calibrator and control).
If there is any damage on the package, do not use it Read the user manual carefully before use, do not use the expired assay kit Do not mix different lot reagents.
All samples should be considered epidemic material, please dispose of them in accordance with the laboratory working standard of infectious diseases.
Take the necessary protective measures to prevent users from becoming infected during operation.
This kit contains components classified according to regulation (EC) 1272/2008 as follows:

Danger:
H290 It can be corrosive to metals.
H319 It causes severe irritation to the eye.
H360FD It can harm fertility. It can harm the unborn child.
Prevention:
P201 Obtain special instructions before use.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.
Answer:
P308 + P313 If exposed or alarmed: Seek medical advice/help.
P337 + P313 If eye irritation persists: Seek medical advice/help.
P390 Absorb spillage to prevent material damage.
Disposal:
P501 Dispose of the contents/container at an approved waste disposal facility.

Use of reagents

Ready to use.

Storage and stability

All components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8ºC, protected from light and contamination is avoided during their use.
Do not use reagents after the expiration date. Signs of reactive deterioration: Presence of particles and turbidity.

Sample collection and preparation

Use only suitable tubes and collection containers to collect and prepare specimens. Only the samples listed below have been tested and found acceptable.
Serum/Plasma: Liheparin and K2EDTA plasma
Centrifuge samples containing precipitate before performing the test. For detailed information on possible sample interactions, see the limitations and interactions section. Sample stability claims were determined by the manufacturer based on experimental data or reference literature and only for the temperatures/time frames specified in the method sheet. To determine the specific stability criteria for the laboratory, all available

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It is the responsibility of each laboratory to use references and/or their own work.

Stability:

2 days at 15-25 °C
7 days at 2-8 °C
(-15)-(-25) 6 months at °C

Required Materials (not included in the kit)

1. Cat# 24BIO01-DC Meditest Diachem Calibrator
2. Cat# 24BIO01-DQ Meditest Diacheck Control L1
3. Cat# 24BIO02-DQ Meditest Diacheck Control L2
4. General laboratory equipment
5. Distilled or deionized water

Working Procedure

If you are using a spectrophotometer to perform this test, work with the following procedure. Ask your representative for the application data for fully automatic devices.

1.Test Conditions:
Wavelength: . 555 nm
Cuvette: . . . . . 1 cm light path
Temperature: . 15-25 ºC
2.Set the appliance to zero with distilled water.
3.Place the pipettes in a cuvette.

	Blank	Standard	Sample
R (mL)	1,0	1,0	1,0
Standard (Note 1,2) (µL)	--	5	--
Sample (µL)	--	--	5

4.Stir, incubate at room temperature for **5 minutes**.
5. Read the initial absorbance (A) of the sample, start the stopwatch, and read the absorbances at 1-minute intervals for 3 min.
6. Calculate the difference between absorbances and the average absorbance differences per minute (ΔA/µιν).

Calculation

$$\frac{\text{(A) Sample (A)Sample Blank}}{\text{(A) Calibrator (A)Calibrator Blank}} \times 5 \text{ (standard conc)} = \text{g/dL}$$

Conversion factor: g/dL x 144.9 = µmol/L

Expected values

3.5-5.0 g/dL

Each laboratory should investigate the transferability of the expected values to its patient population and, if necessary, determine its own reference intervals.

Limitations

Hemolysis leads to a decrease in bilirubin values[1,2,3]. A list of drugs and other substances that interact with bilirubin has been reported by Young et al.[4,5].

Performance characteristics

Measuring range: 0.0349-6 g/dL
If the results obtained are greater than the linearity limit, dilute the sample by 1/2 with 9 g/L NaCl and multiply the result by 2.

Precision

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	0,96	2,48	0,96	2,50
SD	0,024	0,51	0,043	0,035
CV (%)	2,52	2,06	4,49	1,41

Sensitivity: 1 g/dL = 0.2003 ΔA/µιν
Accuracy: Results obtained using Meditest reagents (y) showed no systematic differences when compared to other commercial reagents (x). The results obtained using 50 samples are as follows:
Correlation coefficient (r)²: 0.99169
Regression equation y= 0.7177x + 0.05267

The results of the performance characteristics depend on the analyzer used.

References

1. Kaplan A et al. Bilirubin. Clin Chem, The C.V. Mosby, Co., St Louis. Toronto. Princeton 1984; 1238-1241. 436 and 650.
2. Malloy H T. et al. The determination of bilirubin with the photoelectric colorimeter. J. Biol Chem 1937; 112, 2; 481-491.
3. Martinek R. Improved micro-method for determination of serum bilirubin. Clin Chim 1966: Acta 13: 61-170.
4. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.
6. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed AACC 1995.

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