



Bilirubin Jendrassik-Gróf FS*

For in vitro determination of direct and total bilirubin with the Jendrassik-Gróf method on photometric systems

Order Information

Cat. No.	Kit size
1 0849 99 90 336	<ul style="list-style-type: none"> ① 1 x 90 mL Reagent 1 Sulfanilic acid solution ② 1 x 25 mL Reagent 2 Sodium nitrite solution ③ 2 x 100 mL Reagent 3 Accelerator ④ 2 x 100 mL Reagent 4 Fehling solution II

Principle

Bilirubin reacts with diazotized sulfanilic acid to form an azo dye which is red in neutral and blue in alkaline solutions. Whereas the water-soluble bilirubin glucuronides react "directly", the free "indirect" bilirubin reacts only in the presence of an accelerator.

The total bilirubin in serum or plasma is determined using the method of Jendrassik and Gróf by coupling with diazotized sulfanilic acid after the addition of caffeine, sodium benzoate and sodium acetate. A blue azobilirubin is formed in alkaline Fehling solution II. This blue compound can also be determined selectively in the presence of yellow by-products (green mixed coloration) by photometry at 578 nm. The direct bilirubin is measured as the red azo dye at 546 nm using the method of Schellong and Wende without the addition of alkali. The indirect bilirubin is calculated from the difference between the total and direct bilirubin.

Reagents

Concentrations of the Reagents

R1:	Sulfanilic acid	29 mmol/L
	HCl	170 mmol/L
R2:	Sodium nitrite	29 mmol/L
R3:	Caffeine	130 mmol/L
	Sodium benzoate	156 mmol/L
	Sodium acetate	460 mmol/L
R4:	Fehling solution II	
	Potassium sodium tartrate	930 mmol/L
	Sodium hydroxide	1.9 mol/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 15 - 25 °C, protected from light and contamination is avoided. Do not freeze the reagents.

Warnings and Precautions

1. Reagent 1: S24/25: Avoid contact with skin and eyes.
2. Reagent 4 is corrosive. R35: Causes severe burns. S1/2: Keep locked up and out of the reach of children. S24/25: Avoid contact with skin and eyes. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S27/28: After contact with skin, take off immediately all contaminated clothing, and wash immediately with plenty of water. S39: Wear eye/face protection. S45: In case of accident or if you feel unwell, seek medical advice immediately (show this label if possible). S64 If swallowed, rinse mouth with water (only if the person is conscious).
3. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Material required but not provided

NaCl-Solution 9 g/L
General laboratory equipment

Specimen

Serum

It is very important to store the sample protected from light.

Stability [5]:

Direct Bilirubin:	2 days	at	20 - 25 °C
	7 months	at	4 - 8 °C
	6 months	at	-20 °C

if stored immediately. Freeze only once!

Total Bilirubin:	1 day	at	20 - 25 °C
	7 days	at	4 - 8 °C
	6 months	at	-20 °C

if stored immediately. Freeze only once!

Discard contaminated specimens!

Assay Procedure

Optical path 1 cm
Temperature 15 - 25 °C
Measurement Against sample blank

Determination of total bilirubin (see notes 1 and 2)

Wavelength: Hg 578 nm

	Sample blank	Sample
Reagent 2	-	50 µL
Reagent 1	200 µL	200 µL
Reagent 3	1000 µL	1000 µL
Sample	200 µL	200 µL
Mix and allow to stand for 10 to 60 min. at 15 - 25 °C, then add:		
Reagent 4	1000 µL	1000 µL
Mix well and after 5 to 30 min. measure the absorbance of the sample against the blank.		

Calculation

Concentration total bilirubin: [mg/dL] = ΔA x 10.5
[µmol/L] = ΔA x 180

Determination of direct bilirubin (see notes 1 and 2)

Wavelength: Hg 546 nm

	Sample blank	Sample
Reagent 2	-	50 µL
Reagent 1	200 µL	200 µL
NaCl solution	2000 µL	2000 µL
Sample	200 µL	200 µL
Mix immediately and allow to stand at 15 - 25 °C. Exactly 5 min. after the addition of serum measure the absorbance against the sample blank.		

Calculation

Concentration direct bilirubin: $[\text{mg/dL}] = \Delta A \times 14.0$
 $[\mu\text{mol/L}] = \Delta A \times 240$

Notes

- With larger series it is possible to mix 4 parts of sulfanilic acid ❶ and one part of sodium nitrite ❷ in advance. Instead of sulfanilic acid and sodium nitrite 0,2 mL of this diazotization solution is pipetted in the sample. This solution can be used for up to 2 hours at 15 – 25 °C. The conversion factors in this case are: For total bilirubin: 10.3 mg/dL resp 177 mmol/L
For direct reacting bilirubin: 3.7 mg/dL resp. 235 mmol/L
- It is also possible to add only 1.0 mL of isotonic saline to the sample and the blank and then to add 1.0 mL of Fehling solution II ❸ 5 min. after the addition of serum. Mix well and after 5 min. measure the absorbance of the sample against the blank at 578 nm using a 1 cm light path. This factor must then be included in the calculation:
Concentration of direct bilirubin = $\Delta A \times 10.5 [\text{mg/dL}]$
= $\Delta A \times 180 [\mu\text{mol/L}]$
- If using simple measuring devices, other filters or cells, the total and direct bilirubin determination may only be carried out if the conversion factors are determined independently. For the comparison of the bilirubin values from different laboratories, it is recommended to calibrate the determination method for the used laboratory equipment according to standard techniques, and to calculate your own factors.

Controls

For internal quality control DiaSys TruLab N and P controls should be assayed with each batch of samples.

	Cat. No.	Kit size
TruLab N	5 9000 99 10 062	20 x 5 mL
	5 9000 99 10 061	6 x 5 mL
TruLab P	5 9050 99 10 062	20 x 5 mL
	5 9050 99 10 061	6 x 5 mL

Performance Characteristics

Measuring Range

The test has been developed to determine bilirubin concentrations within a measuring range from 0.03 – 10 mg/dL. When values exceed this range samples should be diluted 1 + 1 with NaCl solution (9 g/L) and the result multiplied by 2.

Specificity/Interferences

No interference was observed by hemoglobin up to 400 mg/dL and lipemia up to 800 mg/dL triglycerides for total bilirubin.

Limit of Detection

The lower limit of detection is 0.03 mg/dL.

Precision Bilirubin direct

Intra assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.33	0.00	1.44
Sample 2	0.71	0.01	0.93
Sample 3	0.15	0.00	3.00

Inter assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.77	0.02	2.47
Sample 2	1.99	0.06	2.82
Sample 3	3.44	0.13	3.64

Precision Bilirubin total

Intra assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	0.57	0.007	1.24
Sample 2	1.79	0.008	0.45
Sample 3	3.86	0.030	0.79

Inter assay n = 20	Mean [mg/dL]	SD [mg/dL]	CV [%]
Sample 1	1.37	0.05	3.32
Sample 2	0.76	0.03	3.33
Sample 3	5.96	0.09	1.43

Method Comparison

A comparison of DiaSys Bilirubin Total FS according to Jendrassik-Gróf (y) with a commercially available test (x) using 38 samples gave following results:

$y = 1.02 x - 0.20 \text{ mg/dL}; r = 0.999.$

A comparison of DiaSys Bilirubin Direct FS according to Jendrassik-Gróf (y) with a commercially available test (x) using 27 samples gave following results:

$y = 0.98 x - 0.01 \text{ mg/dL}; r = 0.991.$

Reference Range [1]

Bilirubin total

Neonates	24 h	< 8.8 mg /dL	< 150 $\mu\text{mol/L}$
	2 nd day	1.3 – 11.3 mg /dL	22 – 193 $\mu\text{mol/L}$
	3 rd day	0.7 – 12.7 mg /dL	12 – 217 $\mu\text{mol/L}$
	4 th – 6 th day	0.1 – 12.6 mg /dL	1.7 – 216 $\mu\text{mol/L}$
Children	>1 month	0.2 – 1.0 mg /dL	3.4 – 17 $\mu\text{mol/L}$
Adults		0.1 – 1.2 mg /dL	1.7 – 21 $\mu\text{mol/L}$

Bilirubin direct

Adults and children $\leq 0.2 \text{ mg/dL}$ 3.4 $\mu\text{mol/L}$

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

Literature

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