

# 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"> <li>① 1 x 90 mL Reagent 1 Sulfanilic acid solution</li> <li>② 1 x 25 mL Reagent 2 Sodium nitrite solution</li> <li>③ 2 x 100 mL Reagent 3 Accelerator</li> <li>④ 2 x 100 mL Reagent 4 Fehling's solution II</li> </ul>

## 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's 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. Direct bilirubin is measured as the red azo dye at 546 nm using the method of Schellong and Wende without the addition of alkali. Indirect bilirubin is calculated from the difference between the total and direct bilirubin.

## Reagents

### Concentrations of the Reagents

<b>R1:</b>	Sulfanilic acid	29 mmol/L
	HCl	170 mmol/L
<b>R2:</b>	Sodium nitrite	29 mmol/L
<b>R3:</b>	Caffeine	130 mmol/L
	Sodium benzoate	156 mmol/L
	Sodium acetate	460 mmol/L
<b>R4:</b>	Fehling's 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: Warning. H290 May be corrosive to metals. P234 Keep only in original container. P280 Wear protective gloves/protective clothing/eye protection/face protection. P390 Absorb spillage to prevent material damage.
2. Reagent 4: Danger. H290 May be corrosive to metals. H314 Causes severe skin burns and eye damage. P234 Keep only in original container. P280 Wear protective gloves/protective clothing/eye protection/face protection. P301+P330+P331 If swallowed: rinse mouth. Do not induce vomiting. P303+P361+P353 If on skin (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P304+P340 If inhaled: Remove victim to fresh air and keep at rest in a position comfortable for breathing. P310 Immediately call a poison center or doctor/physician. P390 Absorb spillage to prevent material damage.
3. In very rare cases, samples of patients with gammopathy might give falsified results [7].
4. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
5. For professional use only!

## 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

(Refer to note 1)

Wavelength: Hg 578 nm

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

### Calculation

Concentration total bilirubin:  $[\text{mg/dL}] = A \times 10.5$   
 $[\mu\text{mol/L}] = A \times 180$

### Determination of direct bilirubin

(Refer to notes 1 and 2)

Wavelength: Hg 546 nm

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

## Calculation

Concentration of direct bilirubin:  $[mg/dL] = A \times 14.0$   
 $[\mu mol/L] = A \times 240$

## Notes for manual use

- 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, 200  $\mu L$  of this diazotization solution are pipetted into the sample. This solution can be used for up to 2 hours at 15 to 25 °C. The conversion factors in this case are:

For total bilirubin:  $[mg/dL] = A \times 10.3$   
 $[\mu mol/L] = A \times 177$

For directly reacting bilirubin:  $[mg/dL] = A \times 13.7$   
 $[\mu mol/L] = A \times 235$

- It is also possible to measure direct bilirubin at 578 nm. For this purpose, add only 1000  $\mu L$  of isotonic saline to the sample and the blank. Add 1000  $\mu L$  of Fehling's 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 an optical path of 1 cm. This factor must then be included in the calculation:

Concentration of directly reacting bilirubin:  $[mg/dL] = A \times 10.5$   
 $[\mu mol/L] = A \times 180$

## Controls

For internal quality control DiaSys TruLab N and P controls should be assayed. Each laboratory should establish corrective action in case of deviations in control recovery.

	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 to 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, naproxen up to 0.4 mmol/L and lipemia up to 800 mg/dL triglycerides for total bilirubin. For further information on interfering substances refer to Young DS [6].

### 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	0.35	0.01	2.15
Sample 2	1.79	0.01	0.45
Sample 3	3.86	0.03	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:

$$y = 1.01x - 0.08 \text{ mg/dL}; r = 0.999$$

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

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

## Reference Range [1]

### Bilirubin total

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

### Bilirubin direct

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

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

## Literature

- Thomas L ed. Clinical Laboratory Diagnostics. 1<sup>st</sup> ed. Frankfurt: TH-Books Verlagsgesellschaft, 1998:192-202.
- Tolman KG, Rej R. Liver function. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3<sup>rd</sup> ed. Philadelphia: W.B Saunders Company; 1999. p. 1125-77.
- Jendrassik L, Gróf P. Vereinfachte photometrische Methoden zur Bestimmung des Blutbilirubins. Biochem Zeitschrift 1938;297:82-9.
- Schellong G, Wende U. Mikromethode zur Bestimmung des Serumbilirubins aus Kapillarblut bei Neugeborenen. Arch Kinderheilkunde 1960;162:126-35.
- Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1st ed. Darmstadt: GIT Verlag; 2001;p. 18-9.
- Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
- Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. ClinChemLabMed 2007;45(9):1240-1243.

### Manufacturer



DiaSys Diagnostic Systems GmbH  
 Alte Strasse 9 65558 Holzheim Germany