



BILIRUBIN (TOTAL AND DIRECT)

Colorimetric Method - JENDDRASSIK & GROF

Cat.No. 101-0154

Size: 100 tests

PRINCIPLE:

In the determination of total bilirubin, bilirubin is coupled with diazotized sulfanilic acid in the presence of caffeine to give an azo dye. No caffeine is added when "direct" bilirubin is determined.

SAMPLE:

Fresh nonhemolyzed serum, EDTA- or heparinized plasma.
Store sample in dark until use.

REAGENTS:

1. Reagent 1 - Sulfanilic acid (1 x 50 ml)
Sulfanilic acid 29 mmol/L
HCl 170 mmol/L
2. Reagent 2 - Sodium nitrite (1 x 4 ml)
Sodium nitrite 25 mmol/L
3. Reagent 3 - Caffeine solution (1 x 100 ml)
Caffeine 260 mmol/L
Sodium benzoate 520 mmol/L
4. Reagent 4 - Tartrate solution (1 x 100 ml)
Tartrate 930 mmol/L
NaOH 1900 mmol/L

All reagents are ready for use. Stability up to expiry date stated when stored at +2 °C to +8 °C.
Always keep bottles tightly closed.

PROCEDURE:

- Wavelength: 546 nm
1. for total bilirubin Hg 578 nm (560-600)
 2. for "direct" bilirubin Hg 546 nm (530-555)
- Cuvette: 1 cm light path
Temperature: 20 °C - 25 °C
Zero: against sample blank

1. Total bilirubin

Pipette into test tubes:		
	Sample	Sample blank
Reagent 1	200 µl	200 µl
Reagent 2	50 µl	-
Reagent 3	1000 µl	1000 µl
Serum, plasma	200 µl	200 µl
Mix and allow to stand for at 10 – 60 minutes.		
Reagent 4	1000 µl	1000 µl
Mix, let stand 5 – 30 min. Read the absorbance of sample against sample blank.		

"Direct" bilirubin

Pipette into test tubes:		
	Sample	Sample blank
Reagent 1	200 µl	200 µl
Reagent 2	50 µl	-
NaCl 0.9%	2000 µl	2000 µl
Serum, plasma	200 µl	200 µl
Mix, let stand to exactly 5 min. read the absorbance of the sample against sample blank.		

CALCULATION:

	Total bilirubin	"Direct" bilirubin
Wavelength	Hg 578 nm	Hg 546
Factor:		
c (µmol/L)	185	234
c (mg/dl)	10.8	13.7

EXPECTED VALUES:

Total bilirubin up to 17 µmol/L (1 mg/dl)
Direct bilirubin up to 4.3 µmol/L (0.25 mg/dl)

LINEARITY: up to 425 µmol/L (25 mg/dl)

QUALITY CONTROL:

CONTRO-N 20x5 ml Cat.No. 101-0083
CONTRO-P 20x5 ml Cat.No. 101-0084

REFERENCE:

1. Jeddassik, L., and Grof, P. Biochem. Z. 297:81 (1938).