AGD
CliniPak

BILIRUBIN TOTAL & DIRECT

(Modified Bergh & Muller Single Reagent)

Code	Pack Size
AGDSP-BLM200	TB : 5 X 20 mL DB : 5 X 20 mL
AGDSP - BLM50	TB : 5 x 5mL DB : 5 x 5mL
AGDSP - BLM184	TB : 4 x 23mL DB : 4 x 23mL

INTENDED USE

This reagent kit is intended for in vitro quantitative determination of Total and Direct Bilirubin in serum.

CLINICAL SIGNIFICANCE

Total and Direct bilirubin determination in serum is used for the diagnosis, differentiation and follow-up of jaundice. Generally, jaundice is classified into three types namely hemolytic, hepatic and obstructive.

Increased levels : are observed in hemolytic jaundice due to excessive hemolysis, obstructive jaundice due to regurgitation while hepatic jaundice is associated with increase in both conjugated and unconjugated bilirubin in serum.

PRINCIPLE

Bilirubin reacts with diazotized sulphanilic acid to form an azo compound the color of which is measured at 546nm (530-560 nm) and is proportional of bilirubin. In the presence of accelerator only conjugated bilirubin reacts with Direct Bilirubin. The readings for Total and Direct Bilirubin are taken after 5 min of incubation.The total bilirubin reaction is stable for 10 minutes.

$$\text{Sulphanilic Acid} + NaNO_2 \xrightarrow{HCl} \text{Diazotized sulphanilic Acid}$$

$$\text{Bilirubin} + \text{Diazotized Sulphanilic} \xrightarrow{\text{pH 1.4}} \text{Azobilirubin}$$

PROCEDURE

Reagent + Sample ⟶ Measurement

(300µL) (15 µL) 300sec (546nm)

REAGENT COMPOSITION

Reagent Total Bilirubin

Sulphanilic Acid	≈ 32 mmol/L
Sodium Nitrite	≈ 290 mmol/L
Hydrochloric Acid	≈ 165 mmol/L
DMSO	≈ 7 mmol/L

Reagent Direct Bilirubin

Sulphanilic Acid	≈ 32 mmol/L
Sodium Nitrite	≈ 29 mmol/L
Hydrochloric Acid	≈ 165 mmol/L

REAGENT STORAGE AND STABILITY

The reagent is stable till the expiry date stated on the reagent bottle label, when stored at 2-8°C and protected from sunlight.

SAMPLE

Serum is preferred; plasma with heparin as anticoagulant may be used. Serum or plasma should be separated as early as possible Avoid exposure of samples to direct light during processing and storage.

SHORT NAME	BILIRUBIN T & D
NAME	BILIRUBIN TOTAL & DIRECT
METHOD	MODIFIED BERGH & MULLER
ASSAY	End point (two point reagent blank)
MAIN WAVE	546nm
SUB WAVE	630nm
DECIMAL	2
UNIT	mg/dL
R1 VOLUME	300µL
INCUBATION	36 secs
R2 VOLUME	0
SAMPLE VOLUME	15µL
INCUBATION	0
MIXING SPEED	0
REACTION TIME (Delay)	260 sec
CHECK TIME (Read)	40 sec
LINEAR RANGE	20mg/dL
ABSORBANCE RANGE	0-2.5
CALIBRATION TYPE	SINGLE SPOT LINEARITY
DILUTION RATIO	6
SAMPLE VOLUME	50

BASIC PARAMETER

*Enter calibrator value as per the lot of calibrator and method.

LINEARITY

Reagent is linear up to 20mg/dL. Dilute the sample appropriately and re-assay if Total & direct bilirubin concentration exceeds 20mg/dL. Multiply result with dilution factor.

REFERENCE NORMAL VALUE

Total bilirubin: Adults & children over 10 days : 0.0- 1.0 mg/dL Direct Bilirubin : 0.0 - 0.25mg/dL

QUALITY CONTROL

To ensure adequate quality control,it is recommended that the laboratory should use a normal and abnormal commercial reference control serum.The quality control material is used to check the function of reagents and the machine together.

LIMITATIONS & PRECAUTIONS

- Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
- Storage condition as mentioned on the kit to be adhered.
- Before the assay bring all the reagents to the room temperature.
- Avoid contamination of the reagent during assay process.
- Use clean glassware free from dust and debris.
- Reagent to sample ratio as mentioned above must be strictly adhered, as any changes it affects the factor and result.
- In case reagent comes in contact with eyes,immediately rinse with plenty of water and seek medical advice.

CE | IVD

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