

INTRODUCTION

1. AutoZyme Bilirubin is a reagent set for determination of Total and Direct Bilirubin **based on Jendrassik & Grof method**, using Diazotized Sulphanilic Acid with DMSO as an activator for Total Bilirubin.
2. AutoZyme Bilirubin is a **two reagent system** using one step procedure.
3. AutoZyme Bilirubin is **linear** upto 20 mg%.
4. AutoZyme Bilirubin can be used on any **Colorimeter, Spectrophotometer, Discrete semi-automated and Automated analyzer**. Programme can be designed for any specific analyzer upon request.
5. AutoZyme Bilirubin has **one step reconstitution**.
6. AutoZyme Bilirubin can be determined in just **5 minutes**.
7. AutoZyme Bilirubin method is relatively free of **interference from haemoglobin** and other commonly occurring substances in serum or plasma.
8. This insert covers technical matter for the complete range of AutoZyme Bilirubin kits available viz.:

Product	Pack-Size
i. Bilirubin T & D (Total and Direct) 200	1 x 200 ml
ii. Bilirubin Total 1000	1 x 1000 ml
iii. Bilirubin Direct 1000	1 x 1000 ml

Please read the appropriate matter depending on the kit you have purchased.
NOTE :- T₁ with pink colour

PRINCIPLE

Bilirubin reacts with diazotized sulphanilic acid to produce azobilirubin (violet colour). DMSO catalyzes the formation of azobilirubin from free bilirubin. The violet colour is proportional to bilirubin concentration measured at 546 nm. (530 - 550 nm).

TOTAL BILIRUBIN



DIRECT BILIRUBIN



*DMSO = Dimethyl Sulphoxide

REAGENT STORAGE & STABILITY

The reagent kit should be stored at Room Temperature (25-30°C) and is stable till the expiry date indicated on the label.

The working solution is stable for **2 days** at 2-8°C and for 5 hours at Room Temperature (25-30°C).

COMPONENTS & CONCENTRATION OF WORKING SOLUTION

Component	Concentration	
	Total Bilirubin	Direct Bilirubin
• Sulphanilic Acid	32 mmol/l	32 mmol/l
• Sodium Nitrite	290 mmol/l	29 mmol/l
• Hydrochloric Acid	165 mmol/l	165 mmol/l
• DMSO	7 mmol/l	—

SPECIMEN COLLECTION & PRESERVATION

Blood should be collected in a clean dry container. Although serum is preferred plasma can also be used as sample.

Following anticoagulant can be used for plasma separation :

- EDTA 2 mg/ml of blood
- HEPARIN 200 IU/ml of blood

Bilirubin is light sensitive. Avoid exposure of serum or plasma to direct light. Bilirubin in serum and plasma is stable for a day at 2-8°C or one month at -10°C. Samples should be brought to room temperature before use.

PROCEDURE

- Reaction type End-Point
- Reaction time 5 mins. at R.T. (25-30°C)
- Wavelength 546 nm. (530 - 550 nm.)
- Zero setting with Serum Blank
- Sample volume 0.05 ml (50 µl)
- Reagent volume 1.02 ml
- Factor 20.2
- Linearity 20 mg%

Manual assay procedure

Perform the assay as given below :

TOTAL BILIRUBIN

NOTE :- The reagents T₁, T₂ and sample requires proper mixing.

	Serum Blank	Test
Reagent T1	1.0 ml	1.0 ml
Reagent T2	—	0.02 ml
Serum / Plasma	0.05 ml	0.05 ml

DIRECT BILIRUBIN

	Serum Blank	Test
Reagent D1	1.0 ml	1.0 ml
Reagent D2	—	0.02 ml
Serum / Plasma	0.05 ml	0.05 ml

Mix, and incubate the assay mixture at Room Temperature (25-30°C) for 5 minutes. Read before 8 minutes the absorbance of the test against their respective blanks at 546 nm (530 - 550 nm).

Calculation :

Bilirubin mg% = (Abs. of Test - Abs. of Blank) x 20.2
(Total or Direct)

