Pariksha Biotech
A game changer in IVD

Pediatric Bilirubin (Total & Direct) Estimation Kit

High -Q Pediatric Bilirubin-(Total & Direct) (DCA Method)

HIGH Q
Command on Quality

Intented Use: Kit for the quantitative determination of of Pediatric Total Bilirubin and Direct Bilirubin in serum or Plasma.

Clinical History:
Red blood cells at the end of their circulating life are broken down in the reticulo-endothelial system, mainly the spleen. The resulting heme, once the iron is removed, is then converted to bilirubin. This process accounts for about 80% of the 300 mg (500 µmol) of bilirubin formed daily. Other sources of bilirubin include the breakdown of myoglobin and cytochromes and the catabolism of immature red blood cells in the bone marrow.Once formed, bilirubin is transported to the liver bound to albumin as it is water insoluble. This fraction of bilirubin is referred to as indirect or unconjugated bilirubin. In the liver, bilirubin is conjugated to glucuronic acid (mono- and diglucuronides) to form conjugated bilirubin by the enzyme uridyl diphosphate glucuronyl transferase. Conjugated bilirubin or direct bilirubin is excreted via the biliary system into the intestine, where it is metabolized by bacteria to a group of products known collectively as stercobilinogen. Elimination is almost complete and serum levels are normally negligible. Neonatal bilirubin quantitation is used to monitor diseases causing jaundice in the newborn, chiefly erythroblastosis fetalis (also called hemolytic disease of the newborn or HDN). HDN is associated with various Rh antibodies, ABO incompatibility, and antibodies involving additional blood groups including Kidd, Kell, and Duffy. The average full-term newborn infant has a peak serum bilirubin concentration of 5 to 6 mg/dL (86 to 103 µmol/L). Exaggerated physiologic jaundice occurs at values above this threshold (7 to 17 mg/dL [104 to 291 µmol/L]). Serum bilirubin concentrations higher than 17 mg/dL in full-term infants are no longer considered physiologic, and a cause of pathologic jaundice can usually be identified in such infants. The primary concern with respect to exaggerated hyperbilirubinemia is the potential for neurotoxic effects, but general cellular injury also occurs. The term “kernicterus” was introduced in the early 1900s to refer to the yellow staining of the basal ganglia observed in infants who died with severe jaundice.Additional causes of neonatal jaundice are hematoma/hemorrhage, hypothyroidism, Crigler-Najjar syndrome, obstructive jaundice, galactosemia, sepsis, syphilis, toxoplasmosis, cytomegalovirus, rubella, glucose-6-phosphate dehydrogenase (G-6-PDH) deficiency, pyruvate kinase deficiency, and spherocytosis.

Specimen Collection and Handling:
Suitable Specimens Serum and plasma are acceptable specimen
❶ **Serum:** Use serum collected by standard venipuncture techniques into glass or plastic tubes with or without gel barriers. Ensure complete clot formation has taken place prior to centrifugation. When processing samples, separate serum from blood cells or gel according to the specimen collection tube manufacturer’s instructions. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may take longer to complete their clotting processes. Fibrin clots may subsequently form in these sera and the clots could cause erroneous test results
❷ **Plasma:** Use plasma collected by standard venipuncture techniques into glass or plastic tubes. Acceptable anticoagulants are lithium heparin (with or without gel barrier), sodium heparin. Ensure centrifugation is adequate to remove platelets.

- When processing samples, separate plasma from blood cells or gel according to the specimen collection tube manufacturer’s
- instructions.

NOTE: Umbilical cord specimens should not be used.

Storage and Stability of the reagents:

All the reagents in the kit are stable at Room Temperature until expiry date stated on the labels.

Presentation of the kit:

All the reagents are ready to use and there is no need to prepare working reagents anywhere

Reagent Composition:

Total Bilirubin Reagent:

Sulphanilic Acid	: 80 mMol/L
Concentrated Hydrochloric Acid	: 200 mMol/L
2,4-Dichloroaniline	: 3.0 mMol/L

Direct Bilirubin Reagent:

Sulphanilic Acid	: 30 mMol/L
Concentrated Hydrochloric Acid	: 50 mMol/L

Sodium Nitrite Reagent:

Sodium Nitrite:	: 280 mMol/L

System Parameters for Total Bilirubin (Monochromatic with Sample Blank)

Type of Reaction	:	End Point
Reaction Slope	:	Increasing
Wavelength	:	546 nm
Sample Blank	:	yes
Flowcell Temperature	:	37° C
Incubation time	:	5 min. at R.T.
Factor	:	**49.3 (Pediatric Total Bilirubin)**
Sample Volume	:	25 µl + 25 µl (Sample Blank & Test)
Reagent Volume	:	1.050 ml.
Zero setting with	:	Sample Blank

Test **Procedure for Total Bilirubin Estimation (Monochromatic Method):**

Reagent	Sample Blank	Test (T)
Pediatric Total Bilirubin Reagent	**1.00 ml**	**1.00 ml**
Sodium Nitrite	**------**	**50 µl**
Sample	**25 µl**	**25 µl**

Mix & incubate for 5 mins. at R.T. & read the absorbance of Test against its sample blank at 546 nm.

Calculation:

Pediatric Total Bilirubin (mg/dl)= Abs of Test-Abs of Sample Blank x 49.3

System Parameters for Direct Bilirubin (Monochromatic with Sample Blank)

Type of Reaction	:	**End Point**
Reaction Slope	:	Increasing
Wavelength	:	546 nm
Sample Blank	:	yes
Flowcell Temperature	:	37° C
Incubation time	:	5 min. at R.T.
Factor	:	**26.2 (Pediatric Direct Bilirubin)**
Sample Volume	:	25 µl + 25 µl (Sample Blank & Test)
Reagent Volume	:	1.050 ml.
Zero setting with	:	Sample Blank

Test Procedure for Direct Bilirubin Estimation (Monochromatic Method):

Reagent	Sample Blank	Test (T)
Pediatric Direct Bilirubin Reagent	**1.00 ml**	**1.00 ml**
Sodium Nitrite	**------**	**50 µl**
Sample	**25 µl**	**25 µl**

Mix & incubate for 5 mins. at R.T. & read the absorbance of Test against its sample blank at 546 nm.

Calculation:

Pediatric Direct Bilirubin (mg/dl)= Abs of Test-Abs of Sample Blank x 26.2

Quality Control:

The integrity of the assay should be monitored by the use of control sera (normal and abnormal) with known Pediatric Bilirubin concentrations.

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Reference Values: Pediatric Bilirubin Total

Total Bilirubin Chart for New Born Babies:

A bilirubin chart newborn babies is like a graph or a chart, which can help to measure the levels of bilirubin in a newborn baby's blood. Several health experts are known to use a neonatal bilirubin chart, or an infant bilirubin chart, so that they can keep a track of the amount of bilirubin that is present in the baby's blood. After the information has been analyzed and gathered on a bilirubin chart for newborn children, the doctor can decide whether or not a baby needs to undergo jaundice treatment. The information recorded on a bilirubin chart for infants can also be very useful in identifying the type of treatment that the baby needs to undergo.

According to a bilirubin chart in newborn babies, the normal values that have been highlighted are:

Serum/Plasma	Range (mg/dL)
Premature:	
Up to 24 hours	1.0 to 8.0
Up to 48 hours	6.0 to 12.0
3 to 5 days	10.0 to 15.0
7-14 days	<15.0 mg/dL
Full-term Newborn	
Up to 24 hours	2.0 to 6.0
Up to 48 hours	6.0 to 10.0
3 to 5 days	4.0 to 12.0
7 to14 days	Below 10 mg/dl
15 days to 17 years	≤1.0 mg/dL
≥18 years:	≤1.2 mg/dL

With full term babies, if the bilirubin levels are only slightly higher than the normal values listed in the bilirubin chart for newborn babies, then treatment may not even be required. However, all instances of high levels of bilirubin in a child should be closely monitored by a doctor.

Reference Values: Pediatric Bilirubin Direct

Within 48 hours of life the Pediatric Direct Bilirubin values above 2.0 mg/dL indicates Neonatal Jaundice and should be clinically corellated. Normal Pediatric Bilirubin values should be derived by the individual laboratory though we suggest 0.5 to 2.0 mg/dL

Linearity:

The assay is linear up to 51.00 mg/dl. Samples exceeding linearity should be diluted with normal saline and repeated. Multiply the concentration by the dilution factor

Performance:

1. Comparison:

Testing performed between this and a similar method yielded a coefficient of correlation of 0.987 with a regression equation of y=0.98x + 0.02.

Bibliography:

1. Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry, 2nd ed. Philadelphia, PA: WB Saunders; 1994:1459–68.
2. Jacobs DS, DeMott WR, Grady HJ, et al. Laboratory Test Handbook, 4th ed. Hudson, OH: Lexi- Comp; 1996:86.
3. Dennery PA, Seidman DS, Stevenson DK. Drug therapy: neonatal hyperbilirubinemia. N Engl J Med 2001;344(8):581–90.

Ordering Information

Ref./Cat. No.	Pack Size	Presentation
P-PBIL(TD) - 50	4 x 25 ml	Three Reagents
P-PBIL(TD) - 100	8 x 25 ml	

PRODUCT FEATURES:

Intended to be used only for Pediatric Bilirubin(T & D) Estimation

Liquid stable two reagents (Pediatric Total Bilirubin Reagent, Pediatric Direct Bilirubin Reagent and Sodium Nitrite)

Monochromatic Estimations

Requires only 25 µl + 25 µl sample (Monochromatic Estimations with sample blank)

5 minutes End Point assay.

Measuring Wavelength : 546 nms (Monochromatic)

Linearity : 51 mg/dl. (No sample dilution required up to 51 mg/dl)

Serum or Heparinized Plasma as Specimens

Estmination with fixed factor :
Pediatric Total Bilirubin Monochromatic - 49.3
Pediatric Direct Bilirubin Monochromatic - 26.2

Available as multipurpose reagents and dedicated system packs.

Symbols used with IVD devices

Date of manufacture		Manufactured by	
In vitro diagnostic medical device	IVD	Keep away from sunlight	
Do not freeze		This way up	
Use-by date		Do not use if package is damaged	
Keep dry		Batch Code	LOT
Consult instructions for use		Reagent	R
Consult electronic instructions for use	eIFU Indicator	Calibrator Material	CAL
Catalogue number	REF		
Caution			

eIFU Indicator

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