Reagent kit for the quantitative determination of direct bilirubin in serum.

**Diazo-sulfanilic acid method.**

**Principle**
Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. In the presence of dimethylsulfoxide, total bilirubin reacts with diazotized sulfanilic acid to form azobilirubin. In the absence of dimethylsulfoxide, only the direct bilirubin reacts to give azobilirubin.

**Reference value**
- Serum: direct bilirubin <5.1 µmol/l (<0.3 mg/dl)
- Total bilirubin <17 µmol/l (<1.0 mg/dl)

It is recommended that each laboratory should assign its own normal range.

**Reagents**
- **Direct Bilirubin**
  - Reagent (RD1): Sulfanilic acid 3.2 mmol/l, Hydrochloric acid 165 mmol/l
  - Reagent (R2): Sodium nitrite 8.6 mmol/l

**Sample**
Serum free of haemolysis.

Bilirubin in serum is light sensitive and it is recommended that serum be stored in the dark.

The reagent is not suitable for bilirubin determination of infants.

**Procedure**
- All reagents are ready for use.
- Avoid direct exposure to light!

**Preparation of working reagent**
Mixing ratio of RD1 and R2: 125 ml/25 ml
Reagents can only be applied with previous mixing!

**Stability of working reagent**
- 20-25°C: 1 day
- 2-8°C: 5 days

If the optical density of working reagent is higher than 0.02 at 546 nm the reagent can not be used.

**Assay Conditions**
- Wavelength: 555 nm (540-560 nm)
- Secondary wavelength: 600 nm
- Temperature: 37°C
- Cuvette: 1 cm light path
- Measure: end point
- Read against: reagent blank

**Pipette into cuvette**

<table>
<thead>
<tr>
<th></th>
<th>Sample</th>
<th>Calibrator</th>
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</thead>
<tbody>
<tr>
<td>Working reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Sample</td>
<td>75 µl</td>
<td>-</td>
</tr>
<tr>
<td>Calibrator</td>
<td>-</td>
<td>75 µl</td>
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</table>

Mix and read the optical density (A) after exactly 3 minutes incubation.

**Calibration**
(37°C, Diazo with Sulfanilic Acid)
- S1: Distilled water
- S2: Diagnosticum DunaCal Cat. No.: Dcal or Randox Calibration Serum Level I or II or Roche C.F.A.S. (Calibrator for automated system)

Calibration is recommended:
- after reagent lot change,
- as required following quality control procedures.

**Quality control**
A quality control program is recommended for all clinical laboratories. The analysis of material in both the normal and abnormal ranges with each assay is recommended for monitoring the performance of the procedure. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Recommended controls: Diagnosticum DunaCont N (Cat. No.: Dcon-N) and DunaCont P (Cat. No.: Dcon-P)

**PERFORMANCES DATA**
The following data were obtained using the Olympus 600 analyzer.

**Linearity**
The test is linear up to 100 µmol/l (5.88 mg/dl) bilirubin concentration (37°C).

**Sensitivity**
It is recommended that each laboratory establishes its own range of sensitivity as this is limited by the sensitivity of the spectrophotometer used. Under manual conditions however, a change of 0.001 Abs is equivalent to 0.575 µmol/l (0.03 mg/dl) Bilirubin concentration at 540 nm.

**Precision**

<table>
<thead>
<tr>
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<th>Reproducibility</th>
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<tbody>
<tr>
<td></td>
<td>Average concentration (µmol/l)</td>
</tr>
<tr>
<td>sample I</td>
<td>13.8</td>
</tr>
<tr>
<td>sample II</td>
<td>57.8</td>
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**Correlation**
Comparative studies were done to compare our reagent with another commercial Bilirubin Direct reagent.
The results from these studies are detailed below.
Correlation coefficient: r=0.9957
Linear regression: y (µmol/l)= 1.05x-0.52
(x= other commercial reagent, y= own reagent).

**Specificity**
Lipid 400 mg/dl, glucose 55.5 mmol/l (1000 mg/dl) and ascorbic acid 2.84 mmol/l (50 mg/dl) don’t interfere with the assay at the given levels.

**Note**
Do not use reagents after the expiry date stated on each reagent container label.
Do not use products, test solutions and reagents described above for any purpose other than described herein

**For in vitro diagnostic use only!**
The following symbols are used on labels

- IVD: For in vitro diagnostic use
- Use by (last day of the month)
- Temperature limitation
- LOT: Batch Code
- REF: Code

**Bibliography**
Hijmans Van den Bergh A. A., Muller P.: Biochem, 77; 90, (1916).