ab235627– Bilirubin (Total and Direct) Colorimetric Assay Kit

For the measurement of Bilirubin concentration in serum.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab235627

Storage and Stability

On receipt entire assay kit should be stored at -20 °C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Bilirubin Reagent 1	2.5 mL	-20°C
Bilirubin Reagent 2	1 mL	-20°C
Catalyst I	15 mL	-20°C
Total Bilirubin Probe	10 mL	-20°C
Direct Bilirubin Probe	20 mL	-20°C
Bilirubin Standard	2 x 200 µL	-20°C
DMSO I	3.5 mL	-20°C

PLEASE NOTE: DMSO I was previously labelled as DMSO (Anhydrous) and Catalyst I as Catalyst. The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer or plate reader

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Bilirubin Reagent 1: Ready to use as supplied. Warm to room temperature (RT) before use.

Bilirubin Reagent 2: Ready to use as supplied. Light sensitive. Warm to room temperature (RT) before use.

Catalyst I: Ready to use as supplied. Warm to room temperature (RT) before use.

Total Bilirubin Probe: Ready to use as supplied. Warm to room temperature (RT) before use.

Direct Bilirubin Probe: Ready to use as supplied. Warm to room temperature (RT) before use.

Bilirubin Standard: Ready to use as supplied. Warm to room temperature (RT) before use.
Δ Note: *Aliquot into amber vials and store at -20°C.*

DMSO I: Ready to use as supplied. Warm to room temperature to liquefy completely before use.
Δ Note: *All incubations are performed at RT (~25 °C), protected from light.*

Assay Protocol

Sample Preparation:

1) Add 2-50 µl of undiluted serum to desired well(s) in a 96-well plate.
2) Adjust the volume to 50 µl/well with 50% DMSO I (mix 500 µl 100% DMSO I (provided) and 500 µl ddH2O for about 20 wells).

Δ Note: *Bilirubin concentration varies over a wide range depending on the patient's age, gender, and pathological conditions. In healthy patients, bilirubin concentrations (in mg/dl) are: Total: (0.1- 1.2); Indirect: (0.1- 0.7); Direct: (0.1- 0.4).*
Δ Note: *For Unknown Samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the Standard Curve range.*

Standard Curve Preparation

1) Dilute 50 µl of 0.2 µg/µl Bilirubin Standard with 50 µl 100% DMSO I (provided).
2) Add 0, 2.5, 5, 10, 20, 40 µl of the diluted Bilirubin Standard (0.10 µg/µl) into a series of wells in a 96-well plate to generate 0, 0.25, 0.5, 1, 2, and 4 µg of Bilirubin/well.
3) Adjust the final volume to 50 µl/well with 50% DMSO I.

Reaction Mix:

Mix enough reagents for the total number of well(s) to be assayed including Standards, Samples, and Background Controls just before starting the assay.

Item	Reaction Mix	Background Reaction Mix
Bilirubin Reagent 1	20 µl	20 µl
Bilirubin Reagent 2	5 µl	—
ddH2O	—	5 µl

Direct Bilirubin Assay:

1) Add 100 µl of 50% DMSO I to each well and mix well. Add 25 µl of Reagent Mix to each well, mix and incubate for at least 30 min at RT, protected from light.
2) Add 75 µl of Direct Bilirubin Probe to all Standards, Samples, and Background Control wells.
3) Incubate the plate for 15 min at RT, protected from light and record the endpoint absorbance at 550 nm in a plate reader.

Total Bilirubin Assay:

1) Add 100 µl of Catalyst I to each well, mix well and incubate for at least 15 min at RT, protected from light.
2) Then add 25 µl of Reagent Mix to each well as shown above, mix and incubate for 15 min at RT, protected from light.
3) Add 75 µl of Total Bilirubin Probe to all Standards, Samples, and Background Control wells. Mix well and incubate the plate for 15 min at RT, protected from light. Record the endpoint absorbance at 600 nm on a plate reader.

Calculation:

1) Subtract 0 Total Bilirubin Standard reading from all Standard readings.
2) Plot the Linear Total Bilirubin Standard Curve. If the Sample Background Control is significant (i.e. if Sample Background Control for Total Bilirubin/Direct Bilirubin has intrinsic high absorbance at 600/550 nm), then subtract Sample Background Control reading from Sample readings.
3) Apply the corrected OD to the Total Bilirubin Standard Curve to get B µg of Total Bilirubin in the Sample well. Likewise, apply the corrected Sample OD to the Direct Bilirubin Standard Curve to get B µg of Direct Bilirubin in the Sample well.

Sample Total or Direct Bilirubin Concentration (C) = B/V x D µg/µl

Where: **B** is the amount of Total/Direct Bilirubin in the sample well (µg)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor

Total Bilirubin = Unconjugated Bilirubin + Conjugated Bilirubin
Bilirubin Molecular Weight: 584.7 kDa
10 mg/ml ≡ 10 µg/µl ≡ 10000 µg/ml ≡ 1 g/dl

Technical Support

All information / detail is correct at time of going to print.
For all technical or commercial enquiries please go to:

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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)