Unconjugated Bilirubin Quantification Kit

Cat. # 10100 100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Description

Features

The kit is designed for detection of unconjugated bilirubin (UCB) in biological samples. The range of detection is 1 nM-2 µM in 96-well plate format. It can also be adapted for use with 384-well plates or cuvettes.

Principle

This assay is based on ligand-activated fluorescence. Fluorescence resulting from the non-covalent binding of UCB to the fluorescent agent is linearly proportional to the amount of UCB in the sample. Fluorescence can be measured using a standard GFP/FITC set of filters (optimal: 489 nm excitation/ 523 nm emission). The assay is specific for UCB, both albumin-bound and free. The assay is not suitable for conjugated bilirubin and other bilirubin derivatives.

Content

Fluorescent Reagent	5 ml	*store at +4°C*
Sample Dilution Buffer	100 ml	*store at +4°C*
Bilirubin Dilution Buffer	2 ml	*store at -20°C*
UCB Standard Solution (2mM in DMSO)*	4 x 20 µl	*store at -20°C*

Important: Immediately upon receiving, place UCB Standard Solutions to -20°C. Do not open tubes before use.

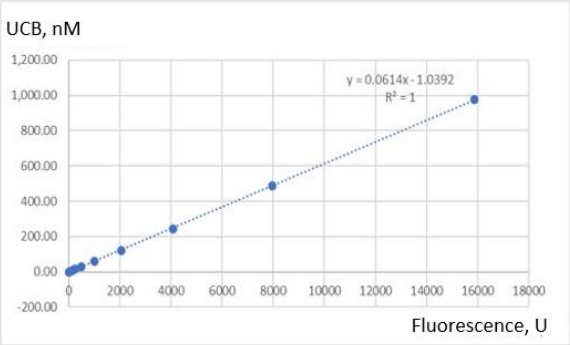
Sample and standard handling

- Store your samples and **UCB Stock Solutions** at -20°C, protected from light.
- Prepare your samples and standards immediately before use. Minimize light exposure.
- Repeated freezing-thawing of the **UCB Standard Solution** will result in UCB degradation of 10% or more per cycle.

Standard preparation

1) Bring **UCB Standard Solution** and **Bilirubin Dilution Buffer** to room temperature.
2) Add 180 µl of **Bilirubin Dilution Buffer** to 20 µl of the provided **UCB Standard Solution** to obtain 200 µM UCB solution. Mix thoroughly.
3) Use **Sample Dilution Buffer** to make further UCB standard dilutions. We recommend two ten-fold serial dilutions followed by several two-fold dilutions. For the standard curve, we recommend 2-1,000 nM range of concentrations (*see example standard curve below*).

Example standard curve for UCB ranging from 4 nM to 1 µM in 96-well plate assay.



Sample preparation

It is important to make sure that UCB concentration in your sample falls into the kit detection range of 1-2,000 nM. Use **Sample Dilution Buffer** to dilute your samples if needed.

- For human blood serum or plasma, we recommend 50x-100x dilutions. If UCB level in your sample exceeds the normal range of 1.7-20 µM (or 0.1-1.2 mg/dL), dilute your sample accordingly.
- For animal blood serum or plasma, we recommend 5x-20x dilutions. UCB level in rodent blood can be in the nanomolar range; therefore, we do not recommend to dilute your sample more than 20x.

Protocol

1) Load 50 µl of your UCB standards, samples, and blanks to 96-well black flat-bottom plate. Use **Sample Dilution buffer** for the blank.
2) Add 50 µl of **Fluorescent Reagent** to each well.
3) Mix, and incubate 15 minutes at room temperature (preferably covered from light). Longer incubation time is acceptable, but it is not recommended to exceed two hours of incubation.
4) Read fluorescence using a GFP/FITC set of filters (optimal: 489 nm excitation/523 nm emission).
5) Use a standard curve to calculate UCB concentration in your samples.

Suggested Applications

The high sensitivity of the method makes it suitable for a range of applications which include, but are not limited to:

In biological fluids:

- Quantification of UCB in small-volume samples;
- Quantification of low levels of UCB in the blood of animals (rodents), which is often below the sensitivity of colorimetric methods;
- Study of a correlation between low serum UCB and different physiological conditions in humans;
- Evaluation of low UCB as a potential diagnostic marker;
- Cerebrospinal fluid analysis.

In cell/tissue culture growth medium:

- Oxidative stress response via activation of heme oxygenase-1;
- Rate of heme catabolism/turnover;
- Deviations in heme synthesis and intracellular transport;
- Red blood cells development, senescence, and heme recycling in in-vitro systems;
- Heme oxygenase activity;
- UCB transport;
- Antioxidant screening.