User's Manual
HBs S Antigen Quantitative ELISA Kit, Rapid-II

BCL-SHP-21

Background

Three types of hepatitis B virus surface antigen (HBsAg), L-, M-, and S-proteins are known. The L-protein is composed of S, Pre-S2, and Pre-S1 domains, and the M-protein is composed of S, Pre-S2 domains, and the S-protein S domain alone. All three surface antigens have S antigen (S-protein) and detection of S antigen has been used for diagnosis of HBV infection because of its high content in serum of HBV-infected patient. The "HB-S Antigen Quantitative ELISA Kit, Rapid-II" can quantitatively detect the activity of S antigen.

Related products

The related products are listed in the below table. We provide a variety of HBV related product.

Product#	Product name (short description)	Content
BCL-AG-01	HBsAg L-protein (recombinant HBsAg with all S- Pre-S1 and Pre-S2 domains)	100 µg
BCL-AGX-02	HBsAg with high antigenic activity, HBsAg-XT (recombinant HBsAg showing extremely high S-antigen activity)	100 µg
BCL-S1HP-01	HBs Pre-S1 Antigen Quantitative ELISA Kit, Rapid (rapid, 90 min, ELISA to determine HBs Pre-S1 antigen)	1 kit (96 tests)
BCL-S2HP-01	HBs Pre-S2 Antigen Quantitative ELISA Kit, Rapid (rapid, 90 min, ELISA to determine HBs Pre-S2 antigen)	1 kit (96 tests)
BCL-AB-01	Anti Pre-S1 antibody, mouse-mono-1 (mouse monoclonal antibody, good for western and ELISA with mouse-mono-2)	100 µg
BCL-AB-02	Anti Pre-S1 antibody, mouse-mono-2 (mouse monoclonal antibody, good for western and ELISA with mouse-mono-1)	100 µg

Other related products are also available, visit our English website; http://www.beacle.com/english/top.html

The principle and outline of assay

The key components of the kit are two types of anti-S antibody and the standard S antigen. So-called sandwich system is employed in this kit, where one type of antibody captures the antigen on the microplate surface, and the captured antigen is detected by another antibody labeled with HRP. Finally the amount of HRP attached was determined using chromogenic substrate.

There are two methods to detect S antigen using the kit, and the approximate times required, excluding times for sample preparation, plate washing, abs measurement etc, are as follows;

- **1-Step method**[1]: Total assay time = 90 min
  ①reaction with capture and detection IgGs(60) → ②chromogenic reaction(30)
- **2-Step method**[2]: Total assay time = 120 min
  ①reaction with capture IgG(60) → ②reaction with detection IgG(30) → ③chromogenic reaction(30)

[note] 1): Assay time can be minimized by shorten the IgG reaction time to 30min, but the sensitivity may be reduced.
2): To get higher signal at low antigen concentration, increase reaction time to with detection IgG to 60 min, or reaction temperature to 37℃. These procedures may cause signal strength too high at higher STD concentration.

The definition of S antigen activity

The strength of S antigen activities of L- and M-protein differs from that of S-protein presumably due to conformational difference. Therefore, in this kit S antigen activity was shown as unit system where 1 nUnit is defined as the activity expressed by 1 ng of the standard antigen that is provided in the kit. The one unit is roughly equal to 0.5 to 1.0 nIU of WHO's standard antigens.

Storage condition and stability

All components can be stored at 4℃. At the condition, all reagents are stable for at least 6 months after shipment.

Materials and Reagents

Kit composition

The kit contains following materials. Please ensure that all materials are provided in the kit.

- Standard S Antigen (lyophilized form, recombinant antigen): 100 ng (1 tube)
- Washing Buffer, 20 x conc.: 40mL (1 bottle)
- Conjugate (HRP-labeled Anti S Detection IgG): 6 mL (1 bottle)
- Substrate Solution A (Chromogenic Reagent A): 6mL (1 bottle)
- Substrate Solution B (Chromogenic Reagent B): 6 mL (light shield 1 bottle)
- Stop Solution: 6.5 mL (1 bottle)
- Assay Plate For S Antigen: 1 plate (pre-coated with anti-S antigen antibody, strip type)
- User's manual

Equipments required for the assay and Reagents not provided by the kit

- Microplate reader (equipped to measure absorbance at 450 nm)
- Micropipettes (for the handling of standard antigen and samples)
- Microtubes (for the handling of standard antigen and samples)
- Plate sealers (or plastic films)
- Multi (8-) channel pipette and its reservoir
- Distilled water or pure water

Assay Procedure

<Preparation before starting>

- **Washing Buffer**: Warm up Washing Buffer (20 x conc.) bottle to room temperature for complete dissolution (chilled concentrated buffer often contains depositions of salts), and dilute the content to 20-fold with distilled or pure water. This makes 400 mL of Washing Buffer ready to use. Storage of diluted Washing Buffer must be at 4℃.
- **Standard antigen**: Pour 1 mL of Washing Buffer into the standard antigen tube and invert it several times for complete dissolving. This makes 100 nUnit/mL (100 ng/mL) antigen solution.

1-Step Method

<①Preparation of standard solutions>

1. Prepare 7 new microtubes and print number from ① to ⑦. As indicated in the below table, transfer 900 µL of Washing Buffer into No.1 microtube, add 100 µL of standard solution (100 nUnit/mL), and mix well. This makes 10 nUnit/mL of STD①. Likewise, prepare the series of standard solutions as indicated in below table.
2. To draw the standard curve, standard solution from STD① to STD⑦ are used.
3. Transfer 400 µL of Washing Buffer into a microtube as the blank.

STD ID	Washing Buffer		Antigen solution for dilution			Final conc.	
STD①	900 µL	+	100 nUnit/mL	100 µL	=	10	nUnit/mL
STD②	500 µL	+	10 nUnit/mL	500 µL	=	5	nUnit/mL
STD③	600 µL	+	5 nUnit/mL	400 µL	=	2	nUnit/mL
STD④	500 µL	+	2 nUnit/mL	500 µL	=	1	nUnit/mL
STD⑤	500 µL	+	1 nUnit/mL	500 µL	=	0.5	nUnit/mL
STD⑥	800 µL	+	0.5 nUnit/mL	200 µL	=	0.1	nUnit/mL
STD⑦	500 µL	+	0.1 nUnit/mL	500 µL	=	0.05	nUnit/mL

<②Preparation of sample solutions>

1. Dilute the sample solution by Washing Buffer so that the expected activity is within the measurable range (0.05～10 nUnit/mL). Sample should be diluted more than 10-fold by Washing Buffer for

accurate measurement.
2. When S antigen activity of a sample can not be estimated, please prepare multiple samples with different dilution factors.
3. Human serum sample should be diluted more than 10-fold by Washing Buffer to prevent the effect of serum components.

[note] When sample contains heavy contaminated proteins, please dilute the sample by Washing Buffer. Use PBS-T (PBS+0.05% Tween-20) when Washing Buffer is not enough for sample dilution.

<③One step reaction of antigen with capture IgG and detection IgG >

1. Take the assay plate out of the aluminum package.
2. In triplicate manner, add 100 µL of sample, standard and blank solutions to each well.
3. Transfer Conjugate（HRP-labeled Detection IgG） reservoir, and by using a 8-channel multi pipette add 50 µL of Conjugate into each well.
4. Cover the plate by a plate sealer, and let it stay at room temperature (around 25°C) for 1 hr.

(go to "④Preparation of Chromogenic solution" for next step)

2-Step Method

<①Preparation of standard solutions>

Prepare standard solutions as indicated in "①Preparation of standard solutions" for 1-Step Method.

<②Preparation of sample solutions>

Prepare sample solutions as indicated in "②Preparation of sample solutions" for 1-Step Method.

<③ 2-Step reaction of antigen with capture IgG and detection IgG >

1. Take the assay plate out of the aluminum package.
2. In triplicate manner, add 100 µL of sample, standard solutions and blank to each well. Incubate at room temperature (around 25°C) for 1 hr.
3. At the end of the reaction of sample with capture IgG, add 50 µL of Conjugate into each well by using 8-channel multi pipette, and incubate for 30 min at room temperature (around 25°C).

(go to "④Preparation of Chromogenic solution" for next step)

<④Preparation of Chromogenic solution>

1. About 10 min before the end of reaction, prepare chromogenic reaction mixture as follows.
2. Transfer 6 mL of Substrate Solution A and 6 mL of Substrate Solution B into a reservoir for 8-channel pipette, and mix well.

[note] The prepared Chromogenic solution decreases the activity by oxidation within fairly short time. So please use the solution within 15 min after preparation.

<⑤Chromogenic reaction>

1. At the end of antibody reaction, remove the reaction solution from each well by decantation followed by gentle tapping on the paper towel.
2. Wash each well with 300 µL/well of Washing Buffer six times with 10 sec interval and remove residual solution by gentle tapping on the paper towel.
3. Add 100 µL of the Chromogenic solution to each well by using an 8-channel pipette, and incubate for 30 min at room temperature in a light shielded container.
4. Stop the reaction by adding 50 µL/well of Stop Solution.

[note] The orange color generated by Chromogenic reaction changes rather quickly. To obtain accurate determinations, it is important to measure the absorbance soon after the addition of stop solution.

<⑥Absorbance measurement and calculation>

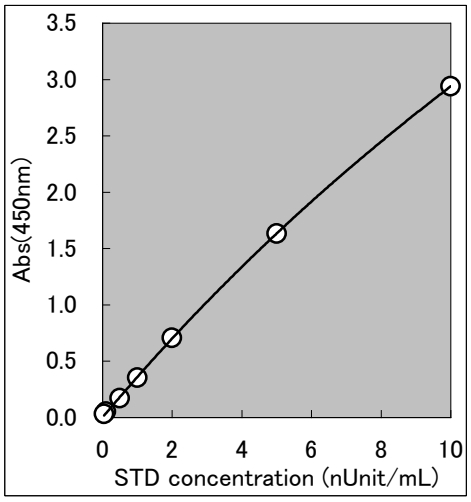
1. Measure the absorbance at 450 nm of each well by using a plate reader.
2. Calculate the specific absorbance of each sample or standard by subtracting the blank value.
3. Calculate the best fit curve between the specific absorbance values and the corresponding standard concentrations using 4 parameters logistic model.
4. The concentration of samples can be calculated from the equation of the best fit curve.
5. The calculated concentrations which are out of the range (<0.05 or >10 nUnit/mL) are not reliable, and not used for determination.

[note] Clean up the bottom of the plate before absorbance measurement to avoid inaccurate measurement.

Example of standard curve

Below is an example of measurements of standard solutions and resulting standard curve.

STD		Abs450nm
ID	nUnit/mL	
STD①	10	2.938
STD②	5	1.631
STD③	2	0.705
STD④	1	0.354
STD⑤	0.5	0.172
STD⑥	0.1	0.051
STD⑦	0.05	0.031



[note] The above data is an example obtained by 2-Step Method in our laboratory, and does not guarantee the performance of the kit. The results may differ due to the difference of the technique and other conditions.

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