

bi-x-act[®]
of FINLAND

Confirm HBsAg

CE IVD

Productcodes: CHBsAg



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CONFIRM HBsAg - A Reagents Kit of Antibody to Hepatitis B Surface Antigen for Confirmatory Tests of HBsAg

Reagents for *in vitro* Diagnosis Only

This reagents kit should be used in combination with an HBsAg ELISA Screening Kit.

NAME & FUNCTION

CONFIRM HBsAg is a test kit for confirmation the existence of HBsAg in the specimens. When CONFIRM HBsAg is used in combination with HBsAg ELISA screening kit, it can confirm the presence or absence of HBsAg in specimens which are positive for HBsAg in screening test.

DESCRIPTION OF THE TEST AND REAGENTS

CONFIRM HBsAg is specially formulated from high potency and strong neutralizing antiserum which contains antibody to HBsAg. CONFIRM HBsAg adopts to the basic principles of the neutralization of antibody with antigen and the competition between neutralizing antibody and ¹²⁵I or enzyme labeled antibody. Even the specimen with very high level of HBsAg still can be accurately confirmed without diluting the specimen in advance. After the first incubation of the procedure, the bead only absorbed part of the HBsAg content in the specimen. Then the unbound HBsAg is removed with the serum by washing the bead. Therefore, the anti-HBs reagent needs to neutralize only that part of HBsAg which has been coupled to the bead. Then only limited quantity of HBsAg left to react with ¹²⁵I or enzyme labeled antibody that added to the test later on.

CONTENTS OF THE KIT

The reagents should be refrigerated at 2 to 8°C. For long period storage, freezing at -20°C is recommended.

1. Solution A (Anti-HBs Positive): One bottle (3 ml), containing anti-HBs (guinea pig) and protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.
2. Solution B (Anti-HBs Negative): One bottle (3 ml), containing normal human serum and protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.

OTHER MATERIALS AND DEVICES NEEDED

1. HBsAg ELISA Screening Kits or other equivalent HBsAg screening kits.
2. Accessories for performing HBsAg test.

TEST PROCEDURE WHEN TEST WITH HBsAg ELISA Screening Kit

1. Bring all reagents and specimens to room temperature (15~30°C) before beginning the assay. Swirl gently before use.
2. Write down the relative numbers of specimens and the wells on the data sheet. For each specimen, four wells are needed and other four wells for positive control. Thus, four pairs are formed. Two of the pairs are marked as AA and the others are BB groups. Three additional wells are needed for negative control into each marked well individually.
3. Add 50 µl of Specimen or Controls into each marked well, individually.
4. Tear off the protective backing of the adhesive slip and press the slip on the plate to seal it.
5. Incubate the plate at Room Temperature (15~30°C) for 20±4 hours.
6. At the end of incubation, wash the plate by following the plate washing procedure of HBsAg ELISA screening kit.
7. Add 50 µl SOLUTION A to AA group and the same amount of SOLUTION B to BB group and the wells with negative control.
8. Cover the plate loosely with the adhesive slip and incubate in a 40°C water bath or incubator for 1 hour.
9. Remove the slip and add 50µl of Anti-HBs • HRPO conjugate solution into each wells (including the Negative Control wells). Seal the plate with a new adhesive slip.
10. Incubate the plate in the 40°C water bath or incubator for 1 hour.

11. Repeat the step 6 to wash the plate.
12. Develop color by following the procedures for HBsAg ELISA screening kit. Stop the reaction by adding 100µl of 2N H₂SO₄.

CALCULATION AND DETERMINATIONS

Follow the calculation methods of HBsAg ELISA screening kit to obtain the result of the test.

1. Calculate the Negative Control mean (NCx). All values of Negative Control should meet the following criteria; otherwise, those tests are invalid.

For HBsAg ELISA screening kit : $NC \leq 0.1$ (Absorbance)

2. Calculate the average test value of group A (Ax) and B (Bx) of each specimen and Positive Control Individually. The mean of B group values should meet the following criteria; otherwise, this specimen is impossible to be confirmed

for HBsAg ELISA screening kit : $Bx \geq NCx + 0.05$

3. Percentage of neutralization can be calculated by the following formula:

$$\frac{Bx - Ax}{Bx - NCx} \times 100\% = \text{Neutralization Percentage}$$

NOTE:

If the OD value of a specimen is greater than 2.0 when tested by HBsAg ELISA screening kit, the specimen should be dilute to 1:10, 1:100 and 1:1000 dilution, then the diluted specimens can be assayed by the confirmatory test.

RESULT INTERPRETATION

If the Neutralizing Percentage of a specimen is EQUAL to or GREATER than 50%, the specimen is confirmed to be POSITIVE for HBsAg and containing HBsAg.

NOTE:

The Positive Control must be confirmed HBsAg POSITIVE at first, otherwise, the whole experiment is considered invalid and should be repeated.