REF Catalogue number

IVD In vitro diagnostic medical device

Manufacturer

Consult instructions for use

Temperature limitation

Use by

LOT Batch code

Sufficient for



# HISCL™ HBsAg Assay Kit

## Identification of the IVD reagent

**HISCL™ HBsAg Assay Kit**

## Intended use

For In Vitro Diagnostic Use
Measurement of Hepatitis B Surface Antigen in serum or plasma

## Development process and characteristics

HBs antigen is the surface antigen of hepatitis B virus (HBV), and its presence indicates HBV infection status. Accordingly, measurement of HBs antigen is extensively used as an important laboratory test for the diagnosis and observation of the clinical course of hepatitis B, for detection of post-transfusion hepatitis and mother-child infection, for prevention of in-hospital infection, etc.
This kit measures HBs antigen based on the chemiluminescence enzyme immunoassay method with CDP-*Star*™ chemiluminescent substrate, and has the following characteristics.

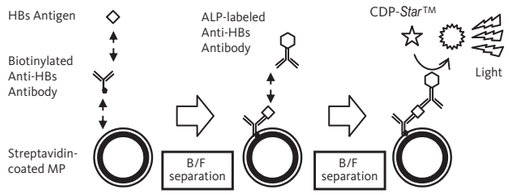
1. This kit is exclusively designed for Sysmex Automated Immunoassay System.
2. Calibrators contain non-infective recombinant antigen, and none of the calibrator components contain human-derived materials.

## Principles of the examination method

This kit measures HBs antigen based on the 2-step sandwich chemiluminescent enzyme immunoassay.

1. Biotinylated anti-HBs monoclonal antibodies (mouse) in R1 reagent specifically react with HBs antigens in the sample, and bind to streptavidin-coated MP (magnetic particles) in R2 reagent.
2. After B/F separation, ALP (alkaline phosphatase)-labeled anti-HBs monoclonal antibodies (mouse) in R3 reagent specifically bind to HBs antigen on MP.
3. After B/F separation, ALP on MP breaks down the CDP-*Star*™ substrate in R5 to an excited intermediate, which produces a luminescent signal.

Because the light production increases in proportion to HBsAg concentration, sample HBsAg concentrations can be obtained with a calibration curve prepared with calibrators.




## Components

This kit consists of the following reagents. Item 4-7 are sold separately.

1. R1 reagent: contains biotinylated anti-HBs monoclonal antibodies (mouse) 1.0 µg/mL
2. R2 reagent
3. R3 reagent: contains ALP-labeled anti-HBs monoclonal antibodies (mouse) 0.3 U/mL
4. HISCL Substrate Reagent Set
   (1) R4 reagent
   (2) R5 reagent: contains CDP-*Star*™:
   Disodium 2-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)-tricyclo[3.3.1.1$^{3,7}$]decan}-4-yl)-1-phenyl phosphate 0.48 mM
5. HISCL Washing solution
6. HISCL HBsAg Calibrator
   (1) HISCL HBsAg C0
   (2) HISCL HBsAg C1
   (3) HISCL HBsAg C2
   (4) HISCL HBsAg C3
   (5) HISCL HBsAg C4
   (6) HISCL HBsAg C5
7. HISCL Diluent

[Note 1] R1 reagent and R3 reagent are provided in a two-in-one reagent container.

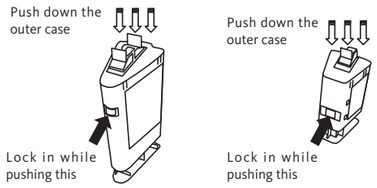
## Warnings and precautions

1. Use the kit according to the method stipulated in the package insert. The reliability of results cannot be guaranteed if the kit is used with a method or for a purpose other than those stipulated.
2. Handle each reagent carefully without generating air bubbles, which may produce incorrect analysis results. If bubbles appear, wait until they disappear.
3. Do not combine reagents from different kits. Do not pool reagents even if the Lot No. of the kits are the same. Use reagents prior to the expiry date. The reliability of results cannot be guaranteed if reagents are used past their expiration date.
4. Avoid eyes and skin contact with R5 reagent as it is an alkaline solution of pH9.6.
5. All Calibrator bottles should be quickly closed after dispensing drops of the Calibrator solution, and then stored at 2-8°C. If bottles are left open, Calibrators may become concentrated due to evaporation, resulting in incorrect calibration.
6. After removing R1-R3 reagent from the analyzer's reagent holder, store them at 2-8°C. Stir R2 reagent according to [Examination procedure] just before you return it to the analyzer. Do not use reagents once they have been frozen, since they may exhibit deterioration.
7. The calibration curves are valid for 30 days. However, even within this period, calibrate again in the following circumstances:
   ・When new R1-R3 reagents with another Lot No. are used.
   ・When quality assurance results are abnormal.
   ・After specified maintenance and/or repair of the analyzer (see analyzer instruction manual).
8. R1-R4 reagents, Calibrators, and Diluent contain sodium azide. Since sodium azide reacts with lead tubing and copper tubing to generate metal azides which can explode, use plenty of water when disposing of it. In case of contact with the eyes, mouth, or hands, carry out emergency treatment such as washing with plenty of water. If necessary, consult a physician.
9. Handle samples carefully. They sometimes contain HBV, HCV, HIV, etc.
10. Do not use the reagent bottles, etc. for other purposes.
11. Use only the reagents (R1-R5 reagents, Calibrators, Diluent and Washing solution) specified in this package insert.
12. Be certain to assemble the reagent containers according to [Examination procedure]. Incorrectly assembled containers may result in device errors or cause evaporation of reagents.
13. Install R4 reagent and R5 reagent carefully to prevent contamination by alkaline phosphatase in saliva or on skin. To prevent absorption of excess $CO_2$, do not remove R5 reagent from the instrument until its bottle is empty and requires replacement.

## Examination procedure

1. Preparation for measurement
   (1) Gently mix R2 reagent thoroughly by circling the container. Look and confirm that the magnetic particles have mixed uniformly.

   (2) First of all push down the outer cases of reagent containers firmly to tear the aluminum seals on the inner bottles.




   (3) Set the containers at the indicated position in the analyzer.
   (4) As a rule dispense 200µL of the sample to reduce the possible effects of evaporation. Refer to the analyzer instruction manual for the minimum volume.
2. Standard assay method *
   (1) Dispense 50µL of R1 reagent and 20µL of the sample into a reaction cuvette, and then incubate for 2 minutes at 42°C.
   (2) Dispense 30µL of R2 reagent into the cuvette, incubate for 1 minute at 42°C, and then perform magnetic separation (contact the magnet with the cuvette, and aspirate liquid).
   (3) Dispense 100-700µL of Washing solution, and then perform magnetic separation. Repeat this procedure 3 times.
   (4) Dispense 100µL of R3 reagent into the cuvette, incubate for 2.5 minutes at 42°C, and then perform magnetic separation.
   (5) Dispense 100-700µL of Washing solution, and then perform magnetic separation. Repeat this procedure 3 times.
   (6) Dispense 50µL of R4 reagent and mix, dispense 100µL of R5 reagent and mix, incubate for 5 minutes at 42°C, and then measure light intensity.
3. Prepare a calibration curve
   (1) Gently stir each of the calibrators (HISCL HBsAg C0-C5) without generating bubbles. Position them according to the analyzer instruction manual.
   (2) Carry out procedures according to the "standard assay method", and then measure light intensity.
   (3) Plot the intensity of the calibrators on the ordinate and the calibrator concentrations on the abscissa, and then prepare a calibration curve. *
4. Sample measurement
   (1) Position a sample according to the analyzer instruction manual.
   (2) Carry out procedures according to the "standard assay method", and then measure light intensity.
   (3) Fit the intensity on the calibration curve to obtain the HBsAg concentration in the sample. *

* The analyzer automatically carries out these procedures.

## Storage and shelf life after first opening

Store at 2-8°C. The shelf life is 30 days after opening.
Do not freeze.

## Control procedure

Analyze control materials as samples according to [Examination procedure].

## Biological reference intervals

Normal sample is negative in this test.

## Interpretation of results

Negative : HBsAg concentration < 0.03 IU/mL
Positive : HBsAg concentration ≥ 0.03 IU/mL

[Note 2] Positive samples with findings of 0.03 - 5.00 IU/mL should be reexamined in duplicate after carrying out centrifugal separation of the sample for more than 10 minutes by 2,000xg. When both of the reexamination results are below 0.03 IU/mL, the evaluation is negative. When both of reexamination results are above 0.03 IU/mL, the evaluation is positive. If a reexamination result does not concur, please check again.

[Note 3] Do not diagnose hepatitis B virus infection based only on test results obtained with this product. Comprehensive assessment is required, with reference to other test results including determination of HBc antibody, etc., and clinical course.

[Note 4] Non-specific reactions can occur in immunoassays. Such reactions are believed to be caused by autoantibodies, insoluble matter (especially fibrin), natural antibodies, etc.

[Note 5] Assess samples with findings above the range of measurement as positive. Use HISCL Diluent in case of dilution test. HISCL Diluent is a product which is sold separately.

## Performance characteristics

1. Sensitivity
   (1) When HISCL HBsAg C0 is analyzed, the light intensity is ≤ 5,000 counts.
   (2) The difference between the light intensity of HISCL HBsAg C0 and the light intensity analyzed using HISCL HBsAg C1 or HISCL HBsAg C2 as a sample shall be 40,000 - 200,000 counts per 1 IU/mL HBsAg.
2. Accuracy
   (1) When HBsAg-negative control serum is analyzed, the result is negative.
   (2) When all HBsAg-positive control sera (L, M, and H) are analyzed, the result is within the labeled concentration ±20%.
3. Reproducibility
   When all HBsAg-positive control sera (L, M, and H) are analyzed simultaneously 10 times, the CV of each result is 15% or less.
4. Measurement range
   0.03 - 2,500 IU/mL

[Note 6] Counts :
Unit of light intensity on Sysmex Automated Immunoassay System.

[Note 7] IU :
International unit of HBsAg concentration based on the WHO standard.

[Note 8] HBsAg-positive control sera:

| | | | |
|---|---|---|---|
| L : | 0.1 | - | 0.5 IU/mL |
| M: | 10 | - | 30 IU/mL |
| H: | 1,000 | - | 2,500 IU/mL |

## Limitations of the examination procedure

1. Limitation-Interference
   Hemoglobin (490 mg/dL or lower), bilirubin (bilirubin F: 18.1 mg/dL or lower, bilirubin C: 19.5 mg/dL or lower), chylo-microns (2,290 formazine turbidity units or lower) and RF (500 IU/mL or lower) each have almost no effect on measurements.(2)
2. Rarely, incorrect results can occur for dilution because of the specimen's properties.

## Reagent preparation

All reagents are ready-to-use.

## Primary sample collection, handling and storage

Human serum or plasma.
1. Plasma should be collected using EDTA or heparin as an anticoagulant. Do not use liquid anticoagulant, since it dilutes samples and causes incorrect results. Do not use sodium fluoride since it yields incorrectly low results.
2. If samples must be stored, freeze at -20°C or lower. Do not repeat freezing and thawing of samples, which may induce formation of particulates and cause incorrect results.



3. Fibrin-clotted samples should be centrifuged at 2,000xg for 10 minutes to remove insoluble matter.

## Disposal procedures

1. Incinerate used sample tubes or reagent bottles, or dispose of them as medical waste or industrial waste according to the rules stipulated for waste materials.
2. When apparatus that has come in contact with any specimens, perform sterilization using one of the following methods:
   - Immerse in 0.05% formalin solution at 37°C for 72 hours or longer.
   - Immerse in 2% glutaraldehyde solution for 1 hour or longer.
   - Immerse in a solution containing 0.1% or more sodium hypochlorite for 1 hour or longer.
   - Autoclave at 121°C for at least 1 hour.

## Literature references

(1) Liver function research group :Journal of Japanese Society of Gastroenterology, **103(12)**, 1403 (2006)
(2) In-house data

## Manufacturer

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## Authorized representative / Distributors

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## Product information

HISCL HBsAg Assay Kit For 100 tests
For 200 tests

## Traceability of values assigned to calibrators

HISCL HBsAg Calibrator has been adjusted by WHO Standard 00/588.

## Date of issue or revision

12/2015

Printed in Japan