REF CU-839-537

HISCL™HBeAg Assay Kit

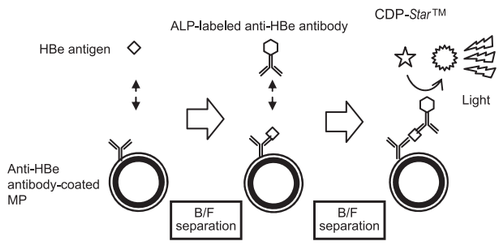
Identification of the IVD reagent
HISCL™ HBeAg Assay Kit

Intended use
For In Vitro Diagnostic Use
Detection of HBe antigen in serum or plasma

Development process and characteristics
An HBe antigen test is commonly performed for diagnosis of HBV infection.
This kit detects HBe antigen (HBeAg) based on the chemiluminescence enzyme immunoassay method with CDP-*Star*™ chemiluminescent substrate.
This kit is exclusively designed for Sysmex automated immunoassay system.

Principles of the examination method
This kit detects HBeAg based on the 2-step sandwich chemiluminescent enzyme immunoassay.
1. HBe antigen in sample specifically react with anti-HBeAg monoclonal antibody (mouse) coated MP (magnetic particles) in R2 reagent.
2. After B/F separation, ALP (alkaline phosphatase)-labeled anti-HBeAg monoclonal antibody in R3 reagent specifically bind HBe antigen on MP.
3. After B/F separation, ALP on MP breaks down CDP-*Star*™ substrate in R5 reagent to an excited intermediate, which produces luminescent signal.

Since the strength of this signal is proportional to HBe antigen concentration, judgment is possible based on the cut-off value established with a sample that contains a known concentration of HBe antigen (HISCL HBeAg Calibrator).



Components
This kit consists of the following reagents. 4 – 6 are products which are sold separately.
1. R1 reagent: reaction buffer
2. R2 reagent: contains magnetic particles coated with anti-HBe antigen monoclonal antibody (mouse) 5 mg/mL
3. R3 reagent: contains ALP-labeled anti-HBe antigen monoclonal antibody (mouse) 0.11 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 HBeAg Calibrator
   (1) HISCL HBeAg NC
   (2) HISCL HBeAg PC

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

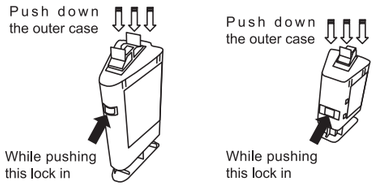
Warnings and precautions
1. Use the kit according to the method stipulated in the package insert. The reliability of results cannot be guaranteed if the kit is used with a method or for a purpose other than those stipulated.
2. Handle each reagent carefully without generating air bubbles, which may produce 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 Nos. of 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 contact of R5 reagent with the skin and eyes, since it is an alkaline solution with pH9.6.
5. All Calibrator bottles should be quickly closed after dispensing drops of Calibrator solution, and then stored at 2-8 °C. If bottles are left open, Calibrators may become concentrated by evaporation, resulting in incorrect calibration.
6. When they are out of the analyzer reagent holder, store R1-R3 reagents 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 frozen, since they may exhibit deterioration.
7. The calibration is valid for 30 days. However, even within this period, calibrate again in the following circumstances:
   (1) When new R1-R3 reagents with another Lot No. are used.
   (2) When quality assurance results are abnormal.
   (3) After specified maintenance and/or repair of the analyzer (see analyzer instruction manual).
8. R1-R4 reagents, and Calibrators contain sodium azide. Since sodium azide reacts with lead tubing and copper tubing to generate metal azides which can explode, use a large quantity of water when disposing of it. In case of contact with the eyes, mouth, or hands, carry out emergency treatment such as washing with a large quantity of water. If necessary, consult a physician.
9. HISCL HBeAg PC contains recombinant HBe antigen, it does not contain human-derived materials.
10. Handle samples carefully. They sometimes contain HBV, HCV, HIV, etc.
11. Do not use the reagent bottles, etc. for other purposes.
12. Use only the reagents (R1-R5 reagents, Calibrators, and Washing solution) specified in this package insert.
13. Be certain to assemble the reagent containers according to [Examination procedure]. Incorrectly assembled containers may result in device errors or cause evaporation of reagents.
14. 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.

REF	Catalogue number		Use by
IVD	In vitro diagnostic medical device	LOT	Batch code
	Manufacturer	Σ	Sufficient for
	Consult instructions for use		
	Temperature limitation		

892

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



(3) Set the containers at the indicated position in the analyzer.
(4) As a rule, dispense 200 µL of sample to reduce possible effects of evaporation. Refer to the analyzer instruction manual for the minimum volume.

2. Standard assay method *
(1) Dispense 20 µL of sample and 50 µL of R1 reagent into a reaction cuvette, and then incubate for 2 minutes at 42 °C.
(2) Dispense 30 µL of R2 reagent into the cuvette, incubate for 1 minute at 42 °C, and then carry out magnetic separation (contact the magnet with the cuvette, and aspirate liquid).
(3) Dispense 100-700 µL of Washing solution, and then carry out 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 carry out magnetic separation.
(5) Dispense 100-700 µL of Washing solution, and then carry out magnetic separation. Repeat this procedure 3 times.
(6) Dispense 50 µL of R4 reagent and mix, dispense 100 µL of R5 reagent and mix, incubate for 5 minutes at 42 °C, and then measure light intensity.

3. Setting cut-off value
(1) Gently stir HISCL HBeAg NC and HISCL HBeAg PC 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) Set the cut-off value based on the light intensity in HISCL HBeAg PC and NC.*

Cut-off value(C) =((P)-(N))/50 + (N)
Where:
P is the light intensity in HISCL HBeAg PC
N is the light intensity in HISCL HBeAg NC

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) Calculate cut-off index (COI) of sample.*

Cut-off index (COI) = (S-N)/(C-N)
Where:
S is the light intensity in sample
C is the cut-off value
N is the light intensity in HISCL HBeAg NC

* The analyzer automatically carries out these procedures.

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

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

Biological reference intervals
Normal sample is negative in this test.

Interpretation of results
The sample is judged positive when the light intensity is above the cut-off value (COI≥1.0) and negative when the light intensity is below the cut-off value (COI<1.0)

[Note 2] When a specimen tests positive for HBeAg or its value is near the cut-off value, it is necessary to collect longitudinal test results and make a comprehensive assessment based on the results of other HBV-related tests and clinical signs and symptoms.
[Note 3] When a patient is suspected of having HBV infection, it is necessary to collect longitudinal test results and make a comprehensive assessment based on the results of other HBV-related tests and clinical signs and symptoms, even if he/she tests negative with this reagent.
[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.

Performance characteristics
1. Sensitivity
(1) When HISCL HBeAg NC is analyzed, the light intensity is ≤ 20,000 counts.
(2) When HISCL HBeAg PC is analyzed, the light intensity is 70,000 – 350,000 counts.
2. Accuracy
(1) When HBe antigen negative control serum is analyzed, the result is negative.
(2) When HBe antigen positive control serum is analyzed, the result is positive.
3. Reproducibility
(1) When HBe antigen negative control serum is analyzed simultaneously 3 times, the results are all negative.
(2) When HBe antigen positive control serum is analyzed simultaneously 3 times, the results are all positive.
4. Measurement range (minimum detectable value)
COI≥1.0

[Note 5] Counts:
Unit of light intensity on Sysmex automated immunoassay system.
[Note 6] HBe antigen negative control serum: HBe antigen negative human serum.
HBe antigen positive control serum: HBe antigen positive human serum.

Limitations of the examination procedure
1. Interference
Hemoglobin (494 mg/dL or lower), bilirubin (bilirubin F: 19.1 mg/dL or lower, bilirubin C: 21.6 mg/dL or lower), and chylomicrons (1,590 formazine turbidity units or lower) each have almost no effect on measurements.(1)
2. Sample with turbidity or hemolysis may not be measured correctly.

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.
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 2,000 xg for 10 min 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. Sterilize any instruments or equipment that have come in contact with specimens 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) In-house data
(2) Selection criterion of hepatitis virus markers in liver diseases (4th edition) Nippon Shokakibyo Gakkai Zasshi **103(12)**:1403 (2006)

Manufacturer
Japan Lyophilization Laboratory
3-1-5 Matsuyama, Kiyose-shi,
Tokyo 204-0022, Japan

Authorized representatives
Asia-Pacific: Sysmex Asia Pacific Pte Ltd.
9 Tampines Grande #06-18, Singapore 528735

Product information
HISCL HBeAg Assay Kit For 50 tests

Traceability of values assigned to calibrators
HISCL HBeAg PC has been adjusted by HBe Referenzantigen 82 (HBe-Ag) made by Paul-Ehrlich-Institute

Date of issue or revision
9/2018