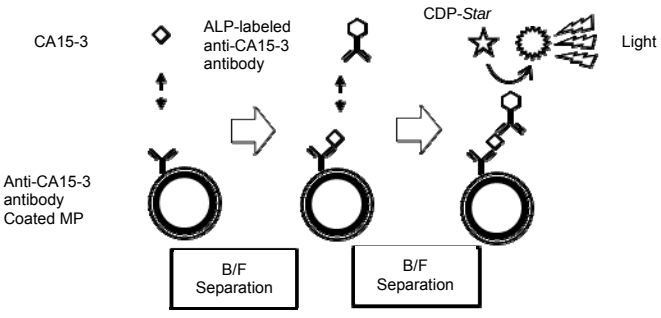
HISCL™ CA15-3 Assay Kit

Identification of the IVD reagent
HISCL™ CA15-3 Assay Kit

Intended use
For In Vitro Diagnostic Use
Measurement of CA15-3 in serum or plasma

Development process and characteristics
CA15-3 is one of the most widely used tumor markers in breast cancer.
This tumor marker is well known to useful for monitoring response to breast cancer treatment and watching for breast cancer recurrence.
This kit measures CA15-3 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. The human-derived material used in HISCL CA15-3 Calibrator.
   C1 to C4 were tested negative for HBs antigen, HCV antibody, HIV-1 antibody, and HIV-2 antibody, but it cannot completely rule out risks for infection. Further, this product was not tested for other viruses. Therefore, please regard this kit as potentially infectious, and handle it carefully.

Principles of the examination method
This kit measures CA15-3 based on the 2-step sandwich chemiluminescent enzyme immunoassay.
1. Anti-CA15-3 monoclonal antibodies (mouse) coated magnetic particles(MP) in R2 reagent specifically react with the CA15-3 in the sample.
2. After B/F separation, alkaline phosphatase (ALP) -labeled anti-CA15-3 monoclonal antibodies (mouse) in R3 reagent specifically bind to the CA15-3 on MP.
3. After B/F separation, ALP on MP breaks down CDP-*Star*® substrate in R5 reagent to an excited intermediate, which produces a luminescent signal.
   Since the strength of the signal is proportional to CA15-3 concentration, CA15-3 concentration in the sample 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 0.1M HEPES buffer
2. R2 reagent: contains Anti-CA15-3 monoclonal antibodies (mouse) coated magnetic particles 5mg/mL
3. R3 reagent: contains ALP-labeled anti-CA15-3 monoclonal antibodies (mouse) 0.28U/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 0.48mM
5. HISCL Washing solution
6. HISCL CA15-3 Calibrator
   (1) HISCL CA15-3 C0
   (2) HISCL CA15-3 C1
   (3) HISCL CA15-3 C2
   (4) HISCL CA15-3 C3
   (5) HISCL CA15-3 C4
7. HISCL Diluent

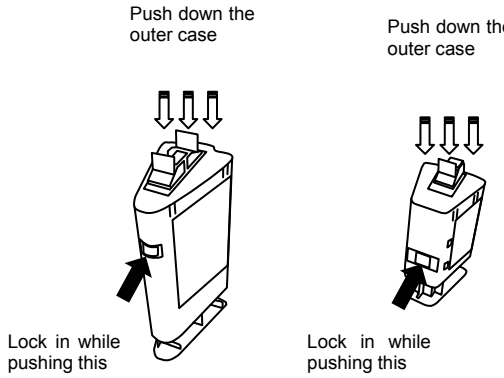
[Note 1] R1 reagent and R3 reagent are provided in a two-in-one reagent container.

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℃. 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℃. 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 is 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 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 100 μL of R1 reagent and 10 μL of the sample into a reaction cuvette, and then incubate for 2 minutes at 42℃.
   (2) Dispense 30 μL of R2 reagent into the cuvette, incubate for 1 minute at 42℃, 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 50 μL of R3 reagent into the cuvette, incubate for 2.5 minutes at 42℃, and then perform magnetic separation.
   (5) Dispense 100-700μL of Washing solution, and then perform 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℃, and then measure light intensity.

3. Prepare a calibration curve
   (1) Gently stir each of the calibrators (HISCL CA15-3 C0-C4) 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 CA15-3 concentration in the sample.*

* The analyzer automatically carries out these procedures.

Storage and shelf life after first opening

Store at 2-8℃. 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

Reference interval: ≤ 23.5U/mL
[Note 2] Reference interval is affected by various factors, setting each laboratory reference range is recommended.

Interpretation of results

1. Do not diagnose cancer based only on test results obtained with this product. Comprehensive assessment is required, with reference to other test results and clinical courses.
2. Non-specific reactions can occur in immunoassays.
   Such reactions are believed to be caused by autoantibodies, insoluble matter (especially fibrin), natural antibodies, etc.
3. Use HISCL Diluent in case of dilution test. HISCL Diluent is a product which is sold separately.

Performance characteristics

1. Sensitivity
   (1) When HISCL CA15-3 C0 is analyzed, the light intensity is ≤ 30,000 counts.
   (2) When HISCL CA15-3 C1 and HISCL CA15-3 C0 are analyzed, the difference of light intensity is 30,000-150,000 counts.
2. Accuracy
   When all CA15-3 control sera (L, M, and H) are analyzed, the result is within the labeled concentration ± 20%.
3. Reproducibility
   When all CA15-3 control sera (L, M, and H) are analyzed simultaneously 10 times, the CV of each result is 15% or less.
4. Measurement range
   0.3 - 1,000 U/mL

[Note 3] HISCL CA15-3 C0: 0U/mL
HISCL CA15-3 C1: 20U/mL
[Note 4] Counts:
Unit of light intensity on Sysmex automated immunoassay system.
[Note 5] CA15-3 control sera
L: 20 - 50 U/mL
M: 80 - 170 U/mL
H: 600 - 1,000 U/mL

Limitation of the examination procedure

1. Limitation-Interference
   Hemoglobin (500 mg/dL or lower), bilirubin (bilirubin F: 18.5 mg/dL or lower, bilirubin C: 20.2 mg/dL or lower)and chylo-microns (1,560 formazine turbidity units or lower)each have almost no effect on measurements.[1)]
2. Cloudy or hemolyzed samples may cause incorrect determination.

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℃ 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 × g 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℃ 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℃ for at least 1 hour.

Literature references

In-house data

Manufacturer

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

Authorized representative / Distributors

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

Product information

HISCL CA15-3 Assay Kit For 50 tests

Traceability of values assigned to calibrators

HISCL CA15-3 Calibrator has been adjusted by in-house standard materials.

Date of issue or revision

10/2015

Printed in Japan