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

REF BE-982-752

HISCL™ AFP Assay Kit

Identification of the IVD reagent
HISCL™ AFP Assay Kit

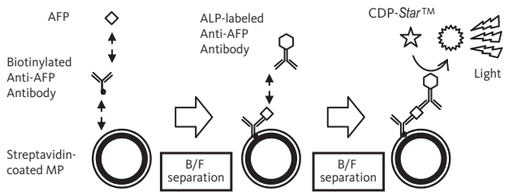
Intended use
For In Vitro Diagnostic Use
Measurement of AFP in serum or plasma

Development process and characteristics
Alfa-fetoprotein (AFP) is the cancer marker. Accordingly, measurement of AFP is extensively used as an important laboratory test for the diagnosis and observation of the clinical course of cancer. This kit measures AFP 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 measures AFP based on the 2-step sandwich chemiluminescent enzyme immunoassay.

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

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



Components
This kit consists of the following reagents. 4 - 7 are products which are sold separately.

1. R1 reagent: contains biotinylated anti-AFP monoclonal antibodies (mouse) 1 µg/mL
2. R2 reagent: contains magnetic particles coated with streptavidin 5 mg/mL
3. R3 reagent: contains ALP-labeled anti-AFP monoclonal antibodies (mouse) 0.2 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 AFP Calibrator
   (1) HISCL AFP C0
   (2)HISCL AFP C1
   (3)HISCL AFP C2
   (4)HISCL AFP C3
   (5)HISCL AFP C4
   (6)HISCL AFP C5
7. HISCL Diluent

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

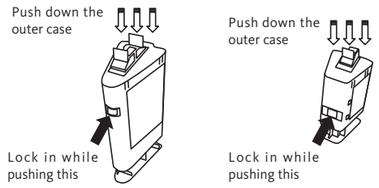
Warnings and precautions

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 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 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 reagent, Calibrators, and Diluent 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 AFP C1 - C5 contains human AFP. Handle with care as potentially infectious, though the material has been checked as negative for HBs antigen, HIV-1 antibody, and HIV-2 antibody.
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.

   (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 the possible effects of evaporation. Refer to the analyzer instruction manual for the minimum volume.
2. Standard assay method *
   (1) Dispense 50 µL of R1 reagent and 10 µL of sample 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 100 µL 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. Prepare a calibration curve
   (1) Gently stir each of the Calibrators (HISCL AFP 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 AFP 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

Reference interval : ≤10 ng/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 judgment 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. Assess samples with findings above the range of measurement 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 AFP C0 is analyzed, the light intensity is ≤10,000 counts.
   (2) When HISCL AFP C1 and HISCL AFP C0 are analyzed, the difference of light intensity is 150,000 - 750,000 counts.
2. Accuracy
   When all AFP-positive control sera (L, M, and H) are analyzed, the result is within the labeled concentration ±15%.
3. Reproducibility
   When all AFP-positive control sera (L, M, and H) are analyzed simultaneously 10 times, the CV of each result is 10% or less.
4. Measurement range
   0.1 - 2000 ng/mL

[Note 3] Counts :
Unit of light intensity on Sysmex Automated immunoassay System.

[Note 4] AFP-positive control sera:
L : 15 - 40 ng/mL
M : 100 - 300 ng/mL
H : 1400 -2000ng/mL

Limitations of the examination procedure

1. Interference
   Hemoglobin (476 mg/dL or lower), bilirubin (bilirubin F: 19.6 mg/dL or lower, bilirubin C: 21.6 mg/dL or lower), and chylomicrons (1,470 formazine turbidity units or lower) each has almost no effect on measurements.
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.After blood sampling, please measure as soon as possible.
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×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. 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) Shirou Iino, Yasuo Endou: Japanese journal of clinical medicine 57 (Suppl.) 439-441 (1999)
(3) Nobuhide Hayashi, et.al: The Journal of Clinical Laboratory Instruments and Reagents 15, 5, 801-811 (1992)

Manufacturer

KAINOS LABORATORIES, INC.
38-18, Hongo 2-chome, Bunkyo-ku, Tokyo
113-0033, Japan

Authorized representative / Distributors

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

Product information

HISCL AFP Assay Kit For 100 tests

Traceability of values assigned to calibrators

HISCL AFP C1 - C5 has been adjusted by in-house standard materials based on WHO 1st International Standard, AFP 72/225.

Date of issue or revision

05/2016

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