REF Catalogue number

IVD In vitro diagnostic medical device

Manufacturer

Consult instructions for use

Temperature limitation

Use by

LOT Batch code

Σ Sufficient for

HISCL™ Insulin Assay Kit

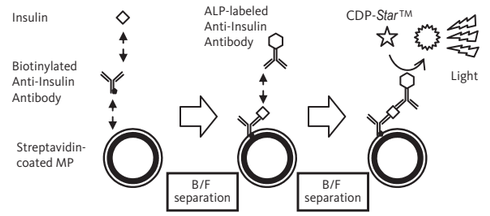
Identification of the IVD reagent
HISCL™ Insulin Assay Kit

Intended use
For in vitro diagnostic use only
Measurement of insulin in serum or plasma

Development process and characteristics
Insulin is a 51-residue peptide hormone that is produced in the pancreas by β-cells of the islets of Langerhans. Insulin is involved in the regulation of carbohydrate, fat and protein metabolism. Lowered levels of insulin cause liver cells to convert glycogen back to glucose and secrete it into the blood.
Insulin also has an effect on small vessel muscle tone, storage and release of (fat) triglycerides and cellular uptake of amino acids and electrolytes. Type 1 diabetes results when the β-cells are destroyed and no longer producing insulin resulting in high glucose levels in the blood. Patients with type 1 diabetes depend on exogenous insulin for their survival because of an absolute deficiency of the hormone; patients with type 2 diabetes have either relatively low insulin production or insulin resistance or both.
This kit measures insulin 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 no calibrator components contain human-derived materials.

Principles of the examination method
This kit measures insulin based on the 2-step sandwich chemiluminescent enzyme immunoassay.
1. Biotinylated anti-insulin monoclonal antibodies (mouse) in the R1 reagent specifically react with insulin in the sample, and bind to streptavidin-coated MP (magnetic particles) in the R2 reagent.
2. After B/F separation, ALP (alkaline phosphatase)-labeled insulin monoclonal antibodies (mouse) in the R3 reagent specifically bind to insulin on MP.
3. After B/F separation, ALP on MP decomposes CDP-*Star*™ substrate in the R5 to an excited intermediate, which produces a luminescent signal.

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



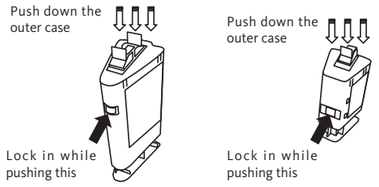
Components
This kit consists of the following reagents. 4 - 7 are separately sold products.
1. R1 reagent: contains biotinylated anti-insulin monoclonal antibodies (mouse) 2.0 µg/mL
2. R2 reagent
3. R3 reagent: contains ALP-labeled anti-insulin monoclonal antibodies (mouse) 0.3 U/mL
4. HISCL Substrate 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 0.48mM
5. HISCL Washing solution
6. HISCL Insulin Calibrator
   (1) HISCL Insulin C0
   (2) HISCL Insulin C1
   (3) HISCL Insulin C2
   (4) HISCL Insulin C3
7. HISCL Diluent

[Note 1] The 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 results of the analysis. 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 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 contact of the 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 dropping 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 out of the reagent holder of the analyzer, store R1-R3 reagents at 2-8°C. Stir the 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 curves are valid for 30 days. However, even within this period, calibrate again in the following cases:
   ・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 instruction manual of analyzer).
8. R1-R4 reagents 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 it. In case of contact with the eyes, mouth, or hands perform emergency treatment such as washing with a large quantity 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 purpose.
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 the reagents.
13. Install the 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 the R5 reagent from the instrument until its bottle is empty and requires replacement.

Examination procedure
1. Preparation for measurement
   (1) Gently mix the R2 reagent thoroughly by circling the container. See and confirm the MP have mixed uniformly. Do not invert the container.

(2) At first, certainly push down the outer cases of reagent containers to tear aluminum seals on the inner bottles.



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

2. Standard assay method *
   (1) Dispense 10µL of sample and 50µL of the R1 reagent into a reaction cuvette, and then incubate for 2 minutes at 42°C.
   (2) Dispense 30µL of the 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 the 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, then perform magnetic separation. Repeat this procedure 3 times.
   (6) Dispense 50µL of the R4 reagent and mix, dispense 100µL of the 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 Insulin C0-C3) without generating bubbles. Position them according to the instruction manual for the analyzer.
   (2) Perform 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 instruction manual for the analyzer.
   (2) Perform procedures according to the "standard assay method", and then measure light intensity.
   (3) Fit the intensity on the calibration curve to obtain the insulin concentration in the sample. *

* The analyzer automatically performs 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: 5.0 - 15.0 µIU/mL[2]

[Note 2] Samples from patients with autoimmune disease frequently exhibit non-specific responses on immunoassay.
[Note 3] Use HISCL Diluent in case of dilution test.

Performance characteristics
1. Sensitivity
   (1) When HISCL Insulin C0 is analyzed, the light intensity is ≤10,000 counts.
   (2) When HISCL Insulin C1 is analyzed, the light intensity is 30,000 - 150,000 counts per 1.0µIU/mL insulin.
2. Accuracy
   When all insulin control sera (L, M, and H) are analyzed, the result is within the labeled concentration±20%.
3. Reproducibility
   When all insulin control sera (L, M, and H) are analyzed simultaneously 10 times, the CV of each result is 15% or less.
4. Measurement range
   0.1 - 500 µIU/mL

[Note 4] HISCL Insulin C0 : 0 µIU/mL
HISCL Insulin C1 : 5 µIU/mL
[Note 5] Counts :
Unit of light intensity on Sysmex Automated Immunoassay System.
[Note 6] IU :
International unit of insulin concentration based on the WHO standard.
[Note 7] Insulin control sera:

L :	1.0	-	5.0µIU/mL
M :	10.0	-	30.0µIU/mL
H :	200.0	-	300.0µIU/mL

Limitations of the examination procedure
1. Limitation-Interference
   Bilirubin (bilirubin F: 18.0 mg/dL or lower, bilirubin C: 19.3 mg/dL or lower), chylomicrons (1,430 formazine turbidity units or lower) and RF (550 IU/mL or lower) each have almost no effect on measurements.[1]
2. In rare cases, incorrect results can occur in diluted samples because of the properties of the specimens.

Reagent preparation
All reagents are ready-to-use.

Primary sample collection, handling and storage
Human serum or plasma.
1. The sample which hemolyzed cannot be used.
2. Plasma should be collected using EDTA, heparin or sodium fluoride as an anticoagulant. Do not use liquid anticoagulant, since it dilutes samples and causes incorrect results.
3. 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.
4. 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 is sterilized, 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) In-house data
(2) LAB DATA TEST SELECTION AND INTERPRETATION 2007-2008, IGAKU-SHOIN

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Product information
HISCL Insulin Assay Kit For 50 tests

Traceability of values assigned to calibrators
HISCL Insulin C1-C3 have been adjusted by in-house standard materials based on WHO Standard 66/304.

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12/2019

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