BIO TECHNOLOGY boditech

Diabetes

ichroma™ Insulin

INTENDED USE

ichroma™ Insulin is a fluorescence immunoassay (FIA) for the quantitative determination of Insulin level in human whole blood/serum/plasma. It is useful as an aid in diagnosis of diabetes mellitus and hypoglycemia.

For *in vitro* diagnostic use only.

INTRODUCTION

Insulin is a protein hormone that regulates the level of sugars (glucose) in the blood and is produced by beta cells on the island of Langerhans in the pancreas; insulin is secreted when blood sugar rises, such as after a meal. When blood glucose levels drop, insulin secretion ceases and glucose is released from the liver into the blood.

Initially, insulin exists as a large molecule called preproinsulin in beta cells; preproinsulin is a single-chain precursor consisting of 110 amino acids. A chain of 24 amino acid of preproinsulin is cleaved to form proinsulin, a precursor to insulin and C-peptides. In proinsulin, chains A and chain B are linked to peptide called a C-peptide. Both insulin and C-peptides are stored and secreted in the secretory granules of pancreatic islet cells in the pancreas.

Decreased or absent insulin activity results in diabetes mellitus, a condition of high blood sugar level (hyperglycaemia). There are two types of the disease. In diabetes mellitus type 1, the beta cells are destroyed by an autoimmune reaction so that insulin can no longer be synthesized or be secreted into the blood. In diabetes mellitus type 2, the destruction of beta cells is less pronounced than in type 1 and is not due to an autoimmune process. Instead, there is an accumulation of amyloid in the pancreatic islets, which likely disrupts their anatomy and physiology.

Type 2 diabetes is characterized by increased glucagon secretion which is unaffected by, and unresponsive to the concentration of blood glucose. But insulin is still secreted into the blood in response to the blood glucose.

Insulin reduces glucose levels in the by using of stimulating glycogenolysis, triglyceride synthesis, and protein synthesis. Failure to stimulate insulin production leads to hyperglycemia without lowering blood glucose levels. Fasting hyperglycemia supports the diagnosis of diabetes mellitus.

Insulin levels can be helpful to evaluate patients with fasting hypoglycemia, to determine insulin resistance in the normal population, and to assess abnormalities in beta cell secretory function.

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized antibodies on a test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for ichroma™ tests to show Insulin concentration in the sample.

COMPONENTS

ichroma™ Insulin consists of 'cartridges', 'detector tubes' and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has streptavidin at the test line, and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector tube has 2 granules containing anti- Insulin-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, anti-Insulin-biotin conjugate and sodium azide as a preservative in MES buffer. All detector tubes are packed in a pouch.
- The detector diluent contains Tris-HCl buffer, Tween 20 and sodium azide as a preservative in Tris-HCl buffer. It is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges or detector tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.

- Used cartridges, detector tubes, detector diluent, capillary tube and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector tube and the detector diluent contain sodium azide (NaN_3), and it may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in ichroma™ Insulin when biotin concentration in the sample was below 10 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.
- **ichroma™ Insulin** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ Insulin** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2EDTA, K_3EDTA, Sodium citrate, Li-Heparin

- **The capillary tube should be used when the following conditions are met.**
  - The capillary tube provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Excess whole blood around the capillary tube should be wiped off.
  - In order to avoid cross-contamination, please do not re-use capillary tube for multiple samples.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Disposable
Detector tube	2 - 30 °C	20 months	Disposable
Detector diluent	2 - 30 °C	20 months	Unopened
		20 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigens to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigens with time and/or temperature may also cause false negative result as it makes antigens unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-181

Components of **ichroma™ Insulin**

- Cartridge box:
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 1
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **ichroma™ Insulin.**

Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™-50 Plus** REF FPRR036
  - **ichroma™ M2** REF FPRR031
- **i-Chamber** REF FPRR009
- **Boditech Insulin Control** REF CFPO-422
- **35μL Capillary tube** REF CFPO-34

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ Insulin** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (whole blood, serum, plasma) may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
- Whole blood sample can be collected using a capillary tube according to below:

① Wear disposable gloves and protective equipment for safety.
② Open the zipper bag which has capillary tubes.
③ Take out the capillary tube and check for damage or contamination.
④ Hold the handle of the capillary tube and touch the surface of blood with the capillary tube.

⑤ Fill it with blood completely.
(Make sure that no air bubbles are present in the capillary tube. Do not get blood on the surface of the capillary tube. If the blood gets on the surface of the capillary tube, remove it gently with gauze.)

TEST SETUP

- Check the contents of **ichroma™ Insulin**: Sealed cartridges, detector tubes, a detector diluent, an ID chip and instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent as well as an ID chip.
- If the sealed cartridge, the detector tube and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the i-Chamber and set the temperature at 25 °C.
- Turn on the instrument for ichroma™ tests.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the instrument for ichroma™ tests operation manual for complete information and operating instructions.**

CAUTION

- To minimize incorrect test results, we suggest that the ambient temperature of the test cartridge should be 25°C during the reaction time after loading sample mixture to the test cartridge.
- To maintain the ambient temperature to 25°C, you can use various devices such as an i-Chamber or an incubator and so on.

TEST PROCEDURE

- **ichroma™ II, ichroma™ M2**

1) Select the sample type (whole blood or serum/plasma) on the screen.
2) Take 150 µL of detector diluent using a pipette and dispense it to the detector tube containing granules. When the granule form is completely dissolved in the tube, it becomes detection buffer.
(The detection buffer must be used immediately. Do not exceed 30 seconds.)
3) Take 35 µL of sample (whole blood/serum/plasma/ control) using a pipette and dispense it to the detector tube.
※ If you use a capillary tube (35 µL), put it into the detector tube after collecting sample.
4) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 20 times.
(The sample mixture must be used immediately. Do not exceed 30 seconds.)
5) Take 75 µL of the sample mixture and dispense it into the sample well of the cartridge.
6) Insert the sample-loaded cartridge into the slot of the i-Chamber or an incubator (25 °C).
7) Leave the sample-loaded cartridge in the i-Chamber or an incubator for 12 minutes.
⚠ Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.
8) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
9) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
(ichroma™ M2 will start the test automatically after inserting.)
10) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
11) Read the test result on the display screen of the instrument for ichroma™ tests.

- **ichroma™ III**

1) The test procedure is same with the 'ichroma™ II test procedure 1) ~ 5)'.
2) Insert the sample-loaded cartridge into the holder of ichroma™ III. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
3) Tap the 'Start' button on ichroma™ III to start the scanning process.
4) The cartridge goes inside and ichroma™ III will automatically start scanning the sample-loaded cartridge after 12 minutes.
5) Read the test result on the display screen of the ichroma™ III.

- **ichroma™-50 plus**

1) Insert the tip array in the tip station.
2) Insert the detector tube in the reagent station and cover the reagent station to hold the detector tubes in place.
3) Open the lid of the detector diluent and insert the detector diluent in the diluent station.
4) Insert the cartridges into the magazine station and close the cover of the magazine station.
5) Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
6) Tap the button located in the upper side of the No. of test cartridge region to select the ID chip that you want to use.
7) When the selected cartridge slot is activated, set the number of the detector tube by tapping.
8) Set the number of pipette tips by tapping.
9) Tap the 'START' button on the left upper of the main screen to start test.
(Please refer to the 'Instrument for ichroma™-50 plus tests Operation Manual' for complete information and operation instructions.)

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays Insulin concentration of the test sample in terms of µIU/mL.
- Reference value: < 25 µIU/mL
- Working range: 2 – 300 µIU/mL

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **ichroma™ Insulin**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the Instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 1.00 µIU/mL
  - Limit of Detection (LoD) 1.25 µIU/mL
  - Limit of Quantitation (LoQ) 2.00 µIU/mL
- **Analytical specificity**
  - **Cross-reactivity**

    Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ Insulin** test results did show the following cross-reactivity data was obtained.

Cross-reactants	Concentration	Cross Reactivity
Proinsulin	4,000 pmol/L	-2.9 %
C-peptide	20,000 pmol/L	-2.73 %
Bovine Insulin	350 pmol/L	23.1 %
Porcine insulin	350 pmol/L	94 %

  - **Interference**

    Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ Insulin** test results did not show any significant interference with these materials.

Interferents	Concentration
Bilirubin unconjugated	684 µmol/L
Cholesterol	10.3 mmol/L
D-glucose	55.5 mmol/L
Hemoglobin	10 g/L
L-Ascorbic acid	298 umol/L
Triglyceride mixture	16.94 mmol/L
EDTA-K2	3.4 µmol/L
EDTA-K3	3.4 µmol/L
Li-Heparin	330 U/dL
Sodium citrate	85 mg/mL

- **Precision**
  - **Single-site study**

    Repeatability (within-run precision)
    within-laboratory precision (Total precision)
    Lot to lot precision

    3 Lots of **ichroma™ Insulin** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
Insulin [µIU/mL]	Repeatability		Within-laboratory precision		Lot to lot pecision	
	AVG [µIU/mL]	CV (%)	AVG [µIU/mL]	CV (%)	AVG [µIU/mL]	CV (%)
10	10.02	5.7	9.98	5.8	10.02	5.8
25	24.91	6.4	25.03	5.8	24.90	5.8
100	99.99	5.8	100.14	5.8	100.17	6.0

  - **Multi-site study**

    Reproducibility

    1 Lot of **ichroma™ Insulin** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

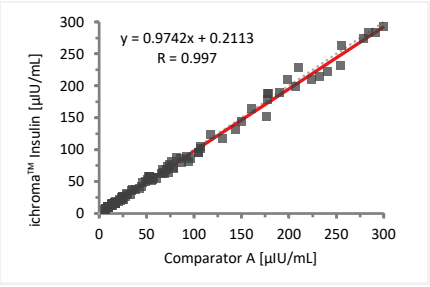
Multi-site study		
Insulin [µIU/mL]	Reproducibility	
	AVG [µIU/mL]	CV (%)
10	9.96	5.6
25	25.09	5.9
100	99.49	5.7

- **Accuracy**

  The accuracy was confirmed by testing 3 different lots of **ichroma™ Insulin**. The tests were repeated 10 times at each concentration of the control standard.

Insulin [µIU/mL]	Lot 1	Lot 2	Lot 3	AVG [µIU/mL]	Recovery (%)
5	4.73	4.78	4.70	4.74	94.7
74	70.91	70.34	71.30	70.85	95.7
98	92.44	91.95	93.81	92.73	94.6
146	139.93	138.86	140.77	139.85	95.8
194	185.56	185.33	187.79	186.22	96.0
290	278.26	273.94	275.27	275.82	95.1

- **Comparability**
  Insulin concentrations of 100 samples were quantified independently with **ichroma™ Insulin (ichroma™ II) and Comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



REFERENCES

1. Andersen, L., Dinesen, B., Jørgensen, P. N., Poulsen, F., & Røder, M. E. (1993). Enzyme immunoassay for intact human insulin in serum or plasma. Clinical Chemistry, 39(4), 578–582.
2. Pu, Y., Zhu, Z., Han, D., Liu, H., Liu, J., Liao, J., … Tan, W. (2011). Insulin-binding aptamer-conjugated graphene oxide for insulin detection. The Analyst, 136(20), 4138.
3. Oh, J., Kim, J. H., & Park, H.-D. (2018). Clinical Utility and Cross-Reactivity of Insulin and C-Peptide Assays by the Lumipulse G1200 System. Annals of Laboratory Medicine, 38(6), 530.
4. Jekarl, D. W., Choi, H., Kim, E. S., Lee, S., Park, H.-I., Kim, M., & Kim, Y. (2019). Analytical evaluation and clinical application of insulin and C-peptide by a whole blood, lateral flow, point of care (POC) assay system. Scandinavian Journal of Clinical and Laboratory Investigation, 1–7.
5. MacDonald, M. J., & Gapinski, J. P. (1989). A rapid ELISA for measuring insulin in a large number of research samples. Metabolism, 38(5), 450–452. doi:10.1016/0026-0495(89)90197-2

Note: Please refer to the table below to identify various symbols

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance, please contact:
Boditech Med Inc.'s Technical Sales
Tel: (+82) -33-243-1400
E-mail: TS@boditech.co.kr

Boditech Med Inc.
43, Geodudanji 1-gil, Dongnae-myeon, Chuncheon-si, Gang-won-do, 24398, Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

EC REP **Obelis s.a**
Bd. Général Wahis 53, 1030 Brussels, Belgium
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net

CE IVD