K-*ASSAY*®

Insulin

For the Quantitative Determination of Human Insulin in Serum and Plasma

Cat. No. KAI-040 and KAI-071

INTENDED USE

For the quantitative determination of human insulin in serum and plasma by immunoturbidimetric assay. FOR *IN VITRO* DIAGNOSTIC USE.

SUMMARY

Insulin is a peptide hormone with approximate molecular weight of 5,800 daltons. Secreted from β cells of the islet of Langerhans in the pancreas, insulin acts to reduce the blood sugar level. Since the blood insulin level reflects the function of β cells, insulin has been widely used as an important diagnostic tool for diabetes mellitus. The **K-*ASSAY*®** Insulin test is a highly specific assay for insulin in serum or plasma.

PRINCIPLE OF TEST

Latex particles coated with antibody specific to human insulin form immune complexes in the presence of insulin from the sample. The immune complexes cause an increase in light scattering that is proportional to the concentration of insulin in the serum or plasma sample. The light scattering is measured by reading turbidity at 600 nm primary, 800 nm secondary. The sample insulin concentration is determined versus insulin calibrators of known concentrations.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent, pH 8.2
Tris(hydroxymethyl)aminomethane (100 mM)

R2: Latex Suspension
Anti-human insulin mouse monoclonal antibody (~1 mg/mL)

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number. Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain < 0.1% w/v sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

Invert R1 and R2 bottles several times gently prior to use. Remove the foam prior to measurement, if necessary.

REAGENT PREPARATION

Reagents are ready to use and do not require reconstitution.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. The reagents can be used until the expiration date. The shelf life of this product is one year from date of manufacture as indicated by the expiration date on the package and bottle labels.

REAGENT STABILITY

Discard reagents if they become contaminated. Evidence of obvious precipitation in reagent 2 (R-2) solution is cause to discard. Opened reagents can be used for 1 month if stored at 2-8°C.

INSTRUMENT

Suitable for two-reagent automated analyzers that can measure a rate reaction at an absorbance of 600 nm (main) and 800 nm (sub). Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and / or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

Serum
Blood should be collected from a patient and the serum separated as soon as possible. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to a plastic tube (not glass) within 2 hours. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2.

Avoid repeated freeze/thaw cycles.

Plasma
Whole blood is collected in sodium citrate, sodium EDTA and sodium fluoride anticoagulant. After collection, immediately centrifuge. In the U.S., follow NCCLS guideline H3-A2. Avoid repeated freeze/thaw cycles.

Serum or plasma may be stored refrigerated (2-8°C) for up to a week.[1] For long-term storage, keep at -20°C or below.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a multi-point calibration method.

PROCEDURE

Materials Supplied

KAI-040 Insulin
Reagent 1 (R-1) Buffer Reagent 1 x 13.5 mL
Reagent 2 (R-2) Latex Suspension 1 x 5 mL

KAI-071 Insulin (L)
Reagent 1 (R-1) Buffer Reagent 2 x 13.5 mL
Reagent 2 (R-2) Latex Suspension 2 x 5 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** Insulin Calibrator, Cat. No. KAI-072C

Purified water

Clinical chemistry analyzer capable of accurately reading at 600 nm (main) and 800 nm (sub), accurately dispensing the required volumes, and maintaining 37°C.

Assay Procedure

An example of automated application:

Sample 12 µL
↓
• ← R1 (Buffer Reagent) 135 µL
↓ 37°C, 5 min.
• ← R2 (Latex Suspension) 50 µL
↓ 37°C, 5 min.
Measure 2 Point End at 600 nm main, 800 nm sub (if available)

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer (Hitachi 917):

			** ANALYZE **			
TEST NAME	: [INS]					
ASSAY/POINT	: [2 POINT END]	[10]	[19]	[34]	[0]	[0]
WAVE (SUB/MAIN)	: [800] [600]					
S. VOL. (NORMAL)	: [12.0] [0.0] [0]					
S. VOL. (DECREASE)	: [ ] [ ] [ ]					
S. VOL. (INCREASE)	: [ ] [ ] [ ]					
DILUENT	: [H2O] [0]					
REAGENT VOL (R1)	: [135] [0] [#] [0]					
REAGENT VOL (R2)	: [0] [0] [ ] [0]					
REAGENT VOL (R3)	: [50] [0] [#] [0]					
REAGENT VOL (R4)	: [0] [0] [ ] [0]					
ABS LIMIT	: [ ] [ ] TWIN TEST : [ ]					
PROZONE LIMIT	: [-32000] [34][LOWER]					
CELL DETERGENT	: [ ]					
	** CALIBRATION **					
CALIB TYPE	: [SPLINE] [ ]					
POINT	: [6] SPAN POINT [0]					
WEIGHT	: [0]					
AUTO CALIBRATION	TIMEOUT CHANGE OVER					
BLANK	: [0] CHANGE LOT: [ ]					
SPAN	: [0] CHANGE BOTTLE: [ ]					
2POINT	: [0]					
FULL	: [0]					
SD LIMIT	: [999] DUPLICATE LIMIT: [10000]Abs					
SENSITIVITY LIMIT	: [0] S1ABS RANGE: [-32000] [32000]					
	** RANGE **					
TEST CODE	: [ ] UNIT: [IU/ml]					
INST. FACTOR (Y=aX + b)	: a= [1.0] b= [0.0]					
	** STANDARD CONCENTRATION **					
STANDARD SOLUTION						
CONC	: [0.0] [*2] [*3] [*4] [*5] [*6]					
POSITION	: [#] [#] [#] [#] [#] [#]					
VOLUME	: [12.0] [12.0] [12.0] [12.0] [12.0] [12.0]					
PRE-DILUENT						
VOLUME	: [0] [0] [0] [0] [0] [0]					
DILUENT	: [0] [0] [0] [0] [0] [0]					
CALIB CODE	: [0] [0] [0] [0] [0] [0]					

Use DI water for standard 1. Use Insulin calibrators 1-5 for standards 2-6.
*2-6: Input concentration of calibrators (using one decimal place [X.X]).
= User Defined

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that a multi-point calibration curve be made using the **K-*ASSAY*®** Insulin Calibrator (KAI-072C). It is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number of other assays being performed. Initially, calibration should be performed each day.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included with every assay performed. The value determined for the controls should fall within the stated limits of the values assigned to the controls. The validity of the assay is in question if the values for the controls generated by the assay's calibration curve do not fall within this range. Recalibrate if the values determined for the controls fall outside the stated range.

LIMITATIONS OF PROCEDURE

The measurable range for insulin is 1.0 to 100 µIU/mL. If the insulin value of a sample is greater than highest calibrator value, dilute 1 part sample with 3 parts isotonic saline and re-assay. Multiply results by 4 to compensate for the dilution.

PERFORMANCE

Sensitivity

When a saline blank is used as a sample, the absorbance change is ≤ 0.0023/min. When a calibrator, having an insulin concentration of around 20 µIU/mL, is assayed, the absorbance (after subtracting the saline blank) is within the range of 0.003 to 0.038/min.

Specificity

When a sample with a known value is assayed, the result is within ± 15% of the assigned value.

Precision

When a sample is assayed 5 times (within-run), the absorbance C.V. is ≤ 10%.

(Within Run)
The following results were obtained on a Hitachi 917 analyzer with human serum:

	Sample I	Sample II	Sample III
N	10	10	10
Mean (µIU/mL)	18.81	27.82	73.17
Std. Dev.	0.224	0.192	0.760
CV	1.19%	0.69%	1.04%

(Between Runs)
The following results were obtained on a Hitachi 917 analyzer with human serum:

	Sample IV	Sample V	Sample VI
N	10	10	10
Mean (µIU/mL)	12.21	25.90	68.74
Std. Dev.	0.530	0.777	1.693
CV	4.34%	3.00%	2.46%

Accuracy / Correlation

A comparison of the **K-*ASSAY*®** Insulin and another company's Insulin EIA was performed with the following results:

Serum Sample
y = 0.8061x + 2.8674
r = 0.997
n = 32
x = another company's Insulin assay
y = **K-*ASSAY*®** Insulin Assay

Plasma Sample
y = 0.8161x + 4.5552
r = 0.986
n = 47
x = another company's Insulin assay
y = **K-*ASSAY*®** Insulin Assay

Assay Range

1.0 to 100 µIU/mL (or value of highest calibration point)

Lower Limit of Detection

The analytical sensitivity is 1 µIU/mL. This means that when saline and serum containing 1 µIU/mL of insulin are tested 10 times, + 2.6 SD of the respective results do not overlap each other.

INTERFERENCE

No cross-reactivity with pro-insulin was observed. Hemoglobin, bile or rheumatoid factor did not interfere with the assay.

Bilirubin F:	No interference up to 19.3 mg/dL
Bilirubin C:	No interference up to 19.9 mg/dL
Hemoglobin:	No interference up to 450 mg/dL
Lipemia:	No interference up to a formazin turbidity of 1,550
RF:	No interference up to 450 IU/mL

PROZONE

No hook effect seen up to at least 1,000 µIU/mL.

EXPECTED VALUES

The expected range for fasting insulin concentration has been reported to be up to 20 to 35 µIU/mL (RIA).[1]

REFERENCES

1. Jacobs DS, *et al.* Ed., Laboratory Test Handbook, 4th Edition. Lexicomp. p. 149 (1996).

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostic Use
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

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