

Insulin

Enzyme immunoassay for the quantitative
determination of Insulin in human serum or plasma

Only for in-vitro diagnostic use

CE

Product Number: DNOV111 (96 Determinations)

CONTENTS

1. INTRODUCTION	3
2. INTENDED USE	3
3. PRINCIPLE OF THE ASSAY	3
4. MATERIALS	3
4.1. REAGENTS SUPPLIED	3
4.2. MATERIALS SUPPLIED	3
4.3. MATERIALS AND EQUIPMENT NEEDED	3
5. STABILITY AND STORAGE	4
6. REAGENT PREPARATION	4
6.1. COATED MICROPLATE	4
6.2. CONJUGATE	4
6.3. INSULIN STANDARDS	4
6.4. TMB SUBSTRATE SOLUTION	4
6.5. STOP SOLUTION	4
6.6 WASH SOLUTION	4
7. SPECIMEN COLLECTION AND PREPARATION	4
7.1. PRECAUTION	4
8. ASSAY PROCEDURE	5
8.1. TEST PREPARATION	5
9. RESULTS	5
9.1. NOTE	5
9.2. CALCULATION	5
9.3. REFERENCE VALUE	6
10. QUALITY CONTROL	6
11. SPECIFIC PERFORMANCE CHARACTERISTICS	6
11.1. PRECISION	6
11.2. SPECIFICITY	6
11.3. SENSITIVITY	6
11.4. CORRELATION WITH RIA	6
12. LIMITATIONS OF THE PROCEDURE	6
13. PRECAUTIONS AND WARNINGS	7
13.1. DISPOSAL CONSIDERATIONS	7
14. LITERATURE	7
15. ORDERING INFORMATION	7

1. INTRODUCTION

Insulin is a polypeptide hormone that regulates carbohydrate metabolism. Apart from being the primary effector in carbohydrate homeostasis, it has effects on fat metabolism and it can change the liver's ability to release fat stores.

Insulin is involved in: control of cellular intake of glucose in muscle and adipose tissue, increase of DNA replication and protein synthesis, modification of the activity of numerous enzyme (allosteric effect), increased glycogen, fatty acid synthesis, amino acid uptake, decreased proteinolysis, lipolysis, gluconeogenesis.

Beta cells release insulin in a glucose-dependent way.

In most humans blood glucose levels varies from about 70 mg/dl to perhaps 110 mg/dl (3.9 to 6.1 mmol/l) except shortly after eating when the blood glucose level rises temporarily. This homeostatic effect is the result of many factors, of which hormone regulation is the most important.

There are several conditions in which insulin disturbance is pathologic: diabetes mellitus, insulinoma, metabolic syndrome and polycystic ovary syndrome. There are two types of diabetes mellitus: type 1 (autoimmune-mediated destruction of insulin producing beta cells in the pancreas resulting in absolute insulin deficiency), and type 2 (multifactor syndrome with combined influence of genetic susceptibility and influence of environmental factors, the best known being obesity, age, and physical inactivity, resulting in insulin resistance in cells requiring insulin for glucose absorption. This form of diabetes is strongly inherited). In both cases the insulin production must be increased by medication or delivering insulin by oral or by intravenous method.

The quantitative determination of insulin can help to determinate the dose to delivery.

2. INTENDED USE

Direct immunoenzymatic colorimetric method for quantitative determination of Insulin in human serum or plasma.

3. PRINCIPLE OF THE ASSAY

The Insulin ELISA test is based on simultaneous binding of human insulin by two monoclonal antibodies, one immobilized on microwell plates and the other conjugates with horseradish peroxidase (HRP).

After incubation, the bound/free separation is performed by a simple solid-phase washing, then the TMB-Substrate solution (TMB) is added. After an appropriate time has elapsed for maximum colour development, the enzyme reaction is stopped and the absorbancies are determined.

The insulin concentration in the sample is calculated based on a series of standard.

The colour intensity is proportional to the insulin concentration in the sample.

4. MATERIALS

4.1. Reagents supplied

- **Coated Mircoplate:** 12 breakpart 8-well snap-off strips coated with monoclonal anti-insulin antibodies; in resealable aluminium foil.
- **Stop Solution:** 1 bottle containing 12 ml sulphuric acid, 0.15 mol/l (avoid any skin contact).
- **Conjugate:** 1 bottle containing 13 ml of horseradish peroxidase labelled monoclonal anti-Insulin antibodies.
- **TMB Substrate Solution:** 1 bottle containing 12 ml 3, 3', 5, 5'-tetramethylbenzidine (H₂O₂-TMB 0.26 g/l) (avoid any skin contact).
- **Wash solution 50x conc.:** 1 bottle containing 20 ml (NaCl 45 g/l and Tween-20 55 g/l)
- **Insulin Standards:** 6 bottles with 2 ml Insulin solution. The concentration is:
 - Standard 0: 0 µIU/ml
 - Standard 1: 5 µIU/ml
 - Standard 2: 25 µIU/ml
 - Standard 3: 50 µIU/ml
 - Standard 4: 100 µIU/ml
 - Standard 5: 300 µIU/ml

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm
- Automatic Dispensers
- Distilled water

5. STABILITY AND STORAGE

The closed reagents are stable up to the expiry date stated on the label when stored at 2...8 °C in the dark. Opened reagents are stable for 60 days when stored at 2...8°C.

6. REAGENT PREPARATION

It is very important to bring all reagents, samples and standards to room temperature (22...28°C) before starting the test run!

6.1. Coated microplate

The ready to use break apart snap-off strips are coated with monoclonal anti-Insulin antibodies. Store at 2...8 °C. Open the bag only when it is at room temperature. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C. Do not remove the adhesive sheets on the unused strips.*

6.2. Conjugate

The conjugate is ready to use.

6.3. Insulin Standards

The standards are ready to use and have the following the concentration:

- Standard 0: 0 µIU/ml
- Standard 1: 5 µIU/ml
- Standard 2: 25 µIU/ml
- Standard 3: 50 µIU/ml
- Standard 4: 100 µIU/ml
- Standard 5: 300 µIU/ml

Once open, the standards are stable 2 months at 2...8 °C. A preservative has been added.

6.4. TMB Substrate Solution

The bottle contains 12 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. *The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.*

6.5. Stop Solution

The bottle contains 12 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

6.6 Wash Solution

Dilute the concentrated wash solution to a volume of 1000 ml with distilled water in a suitable container. For smaller volumes respect the 1:50 ratio. The diluted wash solution is stable for 30 days at 2...8°C.

7. SPECIMEN COLLECTION AND PREPARATION

Follow Good laboratory procedures for handling blood products. For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

The blood should be collected in a venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2...8°C for a maximum period of 5 days. If the specimen (s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing.

When assayed in duplicate, 100µl of the specimen is required.

Patient specimens with Insulin concentrations above 300 µIU/ml may be diluted (for example 1/10 or higher) with standard 0 (Insulin 0 µIU/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.

7.1. Precaution

- The reagents contain Proclin 300^R as preservative.
- Highly hemolysed specimen(s) should not be used.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- This method allows the determination of Insulin from 5 – 300 µIU/ml.
- Avoid exposing TMB/H₂O₂ reagent to direct sunlight, metal or oxidants.
- Plate readers measure vertically. Do not touch the bottom of the wells.

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than one plate is used, it is recommended to repeat the dose response curve. Please allocate at least:

1 well	(e.g. A1)	for substrate blank
2 wells	(e.g. B1+C1)	for standard 0
2 wells	(e.g. D1+E1)	for standard 1
2 wells	(e.g. F1+G1)	for standard 2
2 wells	(e.g. H1+A2)	for standard 3
2 wells	(e.g. B2+C2)	for standard 4
2 wells	(e.g. D2+E2)	for standard 5

It is recommended to determine standards and patient samples in duplicate.

Perform all assay steps in the order given and without any appreciable delays between the steps.

A clean, disposable tip should be used for dispensing each standard and each patient sample.

1. Dispense 50 µl standards and samples into their respective wells.
2. Add 100 µl Conjugate to each well. Leave well A1 for substrate blank.
3. Cover wells with the foil supplied in the kit.
4. **Incubate for 2 hour at room temperature (22...28°C).**
5. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl diluted wash solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.

6. Dispense 100 µl TMB Substrate Solution into all wells.
7. **Incubate for exactly 15 min at room temperature (22...28°C) in the dark.**
8. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution.
Any blue colour developed during the incubation turns into yellow.
9. Measure the absorbance of the specimen at 450 nm.

9. RESULTS

9.1. Note

The OD of calibrator 5 should be ≥ 1.0 .

The optical densities (O.D.s) of some calibrators and samples may be higher than 3.0, in such a case, they could be out of the measurement range of the microplate reader. It is therefore necessary, for O.D.s higher than 3.0, to perform a reading at 405 nm in addition to 450 nm and 620 (reference filter for the subtraction of interferences due to the plastic).

For microplate readers unable to read the plate at 3 wavelengths at the same time,

It is advisable to proceed as follows:

- Read the microplate at 450 nm and at 620 nm.
- Read again the plate at 405 nm and 620 nm.
- Find out the wells whose ODs at 450 nm are higher than 2.0
- Select the corresponding ODs read at 405 nm and multiply these values at 405 nm by the conversion factor 3.0 (where $OD\ 450/OD\ 405 = 3.0$), that is: $OD\ 450\ nm = OD\ 405\ nm \times 3.0$.

Warning: The conversion factor 3.0 is suggested only. For better accuracy, the user is advised to calculate the conversion factor specific for its own reader.

9.2. Calculation

Calculate the mean of the absorbance (E_m) for each point of the standard curve and of each sample.

Use the smoothed cubic spline – preferred – or 4 parameters logistic function as calculation algorithm.

A dose response curve is used to ascertain the concentration of Insulin in unknown specimens.

Record the OD obtained from the printout of the microplate reader.

Plot the OD for each duplicate calibrator versus the corresponding Insulin concentration in µIU/ml on linear graph paper (do not average the duplicates of the calibrators before plotting).

Draw the best-fit curve through the plotted points.

To determine the concentration of Insulin for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in $\mu\text{IU/ml}$) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated).

9.3. Reference value

Insulin values are consistently higher in plasma than in serum; thus, serum is preferred.

The following ranges have been assigned by NovaTec in concordance with the published literature.

These ranges should be used as guidelines only:

Children <12 yrs <10 $\mu\text{IU/ml}$
Adult (Normal) 0.7 – 9.0 $\mu\text{IU/ml}$
Diabetic (Type II) 0.7 – 25 $\mu\text{IU/ml}$

10. QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

If computer controlled data reduction is used to calculate the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

11. SPECIFIC PERFORMANCE CHARACTERISTICS

11.1. Precision

Intra Assay Variation

Within run variation was determined by replicate determination (16x) of two different control sera in one assay. The within assay variability is $\leq 2.3\%$.

Inter Assay Variation

Between run variation was determined by replicate measurements of three different control sera in 2 different lots. The between assay variability is $\leq 12.4\%$.

11.2. Specificity

The cross reaction of the antibody calculated by deriving a ratio between dose of interfering substance to dose of Insulin needed to produce the same absorbance:

Insulin	100%
Proinsulin	n.d.
C-Peptide	n.d.

11.3. Sensitivity

The lowest detectable concentration of Insulin that can be distinguished from standard 0 is 1.03 $\mu\text{IU/ml}$ at the 95 % confidence limit.

11.4. Correlation with RIA

The NovaTec Insulin ELISA was compared to another commercially available Insulin assay. 32 serum samples were compared by linear regression analysis.

The linear regression curve was calculated.

Insulin NovaTec 0 0:97 * reference method + 1.14

$r^2 = 0.892$

12. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values.

13. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV 1+2 antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

WARNING:	In the used concentration Proclin 300 ^R has hardly any toxicological risk upon contact with skin and mucous membranes!
WARNING:	Sulphuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor!

13.1. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

14. LITERATURE

- Eastham R.D: Biochemical Values in Clinical Medicine, 7th Ed. Bristol. England. Jonh Wright & Sons, Ltd; (1985).
- Gerbitz, VKD., Pankreatische B-Zellen Peptide: Kinetik und Konzentration von Proinsulin, Insulin und C- Peptid in Plasma and Urin. Probleme der Messmethoden. Klinische und Literaturübersicht. J. Clin. Chem. Biochem. 18, 313-326 (1980)
- Boehm TM, Lebovitz HE, Statistical analysis of Glucose and Insulin responses to intravenous tolbutamide: evaluation of hypoglycemic and hyperinsulinemic states: Diabetes Care 479-490 (1079)
- Wayne P A, National Committee for Clinical Laboratory Standards. Procedure for the collection of diagnostic blood specimens by venipuncture: approved standards. 4th Ed. NCCLS Document H3-A4, Wayne, PA(1988).
- Turkinton RW, Estkowski A, Link M, Secretion of Insulin dependence of connecting peptide; a predictor of insulin or dependence of obese diabetics. Archive of Internal Med. 142 (1982)
- Sacks BD, Carbohydrates in :Burtis, C.A. and Ashwood, AR (Eds) Tietz Textbook of Clinical Chemistry. 2nd Ed. Philadelphia W. .B. Saunders Co. (1994)
- Kahn CR, Rosenthal AS, Immunologic reactions to insulin, insulin allergy, insulin resistance and autoimmune insulin syndrome. Diabetes Care 2, 283 – 295 (1979)

15. ORDERING INFORMATION

Prod. No.:

DNOV111

Insulin (96 Determinations)

SCHEME OF THE ASSAY

Insulin

Test Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate blank	Stand. 0	Stand. 1	Stand. 2	Stand. 3	Stand. 4	Stand. 5	Sample
Stand. 0	-	50 µl	-	-	-	-	-	-
Stand. 1	-	-	50 µl	-	-	-	-	-
Stand. 2	-	-	-	50 µl	-	-	-	-
Stand. 3	-	-	-	-	50 µl	-	-	-
Stand. 4	-	-	-	-	-	50 µl	-	-
Stand. 5	-	-	-	-	-	-	50 µl	-
Sample	-	-	-	-	-	-	-	50 µl
Conjugate	-	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Cover wells with foil supplied in the kit Incubate for 2 hour at room temperature (22...28°C) Wash each well three times with 300 µl diluted Wash Solution								
TMB Substrate	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Incubate for exactly 15 min at room temperature (22...28°C) in the dark								
Stop Solution	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
Photometric measurement at 450 nm								

NovaTec Immundiagnostica GmbH

Technologie & Waldpark

Waldstr. 23 A6
D-63128 Dietzenbach, Germany

Tel.: +49 (0) 6074-48760 Fax: +49 (0) 6074-487629

Email : info@NovaTec-ID.com

Internet: www.NovaTec-ID.com

DNOV111engl29112010-CR