

# Rat Insulin ELISA Kit

**Cat: OPK10406**

For research use only. Not intended for diagnostic use.

**Sensitivity:** 0.11  $\mu$ IU/mL

**Detection Range:** 5 - 300  $\mu$ IU/mL

## Summary and Explanation

Insulin is the principal hormone responsible for the control of glucose metabolism. It is synthesized in the  $\beta$ -cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form C-peptide and insulin. Both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain and B chain (21 and 30 amino acids respectively). The two chains are linked together by two inter-chain disulphide bridges. There is also an intra-chain disulphide bridge in the A chain. Insulin concentrations are severely reduced in insulin-dependent diabetes mellitus (IDDM) and some other conditions such as hypopituitarism. Insulin levels are raised in non-insulin-dependent diabetes mellitus (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and acromegaly.

## Test Principle

The Rat Insulin ELISA is based on solid phase sandwich ELISA method. The samples and conjugate reagent (anti-Insulin biotin & HRP) are added to the wells coated with Streptavidin. Insulin in the patient's serum binds to the matched pair Abs, forming a sandwich complex and simultaneously the complex is being immobilized on the plate through streptavidin-biotin interactions. Unbound protein and HRP conjugate are washed off, through a washing step. Upon addition of the substrate, the intensity of color is proportional to the concentration of Insulin in the samples. A standard curve is prepared by relating the color intensity to the concentration of Insulin.

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## Kit Components

Reagents	96T
Microwells coated with Streptavidin	12x8x1
Insulin Standards: 6 vials (ready to use)	0.5mL
Insulin Control: 2 vials (ready to use)	0.5mL
Insulin Conjugate Reagent: 1 bottle (ready to use)	12mL
TMB Substrate: 1 bottle (ready to use)	12mL
Stop Solution: 1 bottle (ready to use)	12mL
20X Wash concentrate: 1 bottle	25mL

## Storage and Stability

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose reagent to heat, sun, or strong light.

## Materials Required, Not Supplied

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

## Safety Notes

1. This kit is only used for lab research and development and should not be used for human or animals.
2. Reagents should be regarded as hazardous substances and should be handled carefully and correctly.
3. Gloves, lab coats, and goggles should always be worn to avoid skin and eyes coming into contact with Stop Reagent and TMB. In case of contact, wash thoroughly with water.

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## Sample Collection and Storage

**Serum** - Samples should be collected into a serum separator tube. After clotting for 2 hours at room temperature or overnight at 4°C, and then centrifuging at 1000×g for 20 minutes. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 1000×g and 2-8°C for 15 minutes within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze-thaw cycles.

**Tissue homogenates** -The preparation of tissue homogenates will vary depending upon tissue type.

1. Rinse the tissues in pre-cooled PBS to completely remove excess blood, and weigh them before homogenization.
2. Mince the tissues to small pieces and homogenize them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (PBS can be used as the lysis buffer of most tissues) (w:v=1:9, e.g. 900 µL lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (micro tissue grinders, too).
3. Ultrasound the obtained suspension with an ultrasonic cell disrupter until the solution is clear.
4. Then, centrifuge the homogenates for 5 minutes at 10000×g and collect the supernatant and assay immediately or store in aliquots at ≤ -20°C.

Note: Tissue homogenates are recommended to be tested for protein concentration at the same time to obtain a more accurate concentration of the test substance per mg of protein.

**Cell lysates** - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by pre-cooled PBS gently, and then be detached with trypsin, and collect them by centrifugation at 1000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells 3 times in pre-cooled PBS.
3. Then, resuspend the cells in fresh lysis buffer with concentration of 10<sup>7</sup> cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clear.
4. Centrifuge at 1500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or store in aliquots at ≤ -20°C.

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**Urine** -Collect the first urine of the day (mid-stream) and discharge it directly into a sterile container.

Centrifuge to remove particulate matter, assay immediately or aliquot and store at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Saliva** - Collect saliva using a collection device or equivalent. Centrifuge samples at  $1000\times g$  at  $2-8^{\circ}\text{C}$  for 15 minutes. Remove particulates and assay immediately or store samples in aliquot at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Cell culture supernatants and other biological fluids** - Centrifuge samples at  $1000\times g$  for 20 minutes. Collect the supernatant and assay immediately or store samples in aliquot at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  for later use. Avoid repeated freeze-thaw cycles.

## Notes

1. Samples to be used within 5 days may be stored at  $4^{\circ}\text{C}$ , otherwise samples must be stored at  $-20^{\circ}\text{C}$  ( $\leq 1$  month) or  $-80^{\circ}\text{C}$  ( $\leq 2$  months) to avoid loss of bio-activity and contamination. Avoid repeated freeze-thaw cycles.
2. Sample hemolysis will influence the result, so it should not be used.
3. When performing the assay, bring samples to room temperature.
4. If the concentration of the test material in your sample is higher than that of the Standard product, please make the appropriate multiple dilutions according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio).

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## Reagent Preparation

1. 20X Wash Buffer Concentrate: Prepare 1X wash buffer by adding the contents of the bottle to 475mL of distilled water. Store 1X wash buffer at room temperature.

## Notes

1. After receiving the kit, please store the reagents according to the instructions. The plates can be disassembled to single strips. Please use it in batches on demands.
2. The test tubes, pipette tips and reagents used in the experiment are all disposable and are strictly prohibited from being reused; otherwise, the experiment results will be affected. Kit reagents of different batches cannot be mixed (except TMB, Washing Buffer and Stop Reagent).
3. Bring all reagents to room temperature (18-25°C) before use. If crystals form in the concentrate (20×), it is a normal phenomenon. Heat it to room temperature (the heating temperature should not exceed 40°C), gently Mix until crystals are completely dissolved.
4. During the washing process, the residual washing liquid in the reaction well should be patted dry on absorbent paper. Do not put the paper directly into the reaction well to absorb water. Before reading, pay attention to remove the residual liquid and fingerprints at the bottom, so as not to affect the microplate reader reading.
5. TMB Substrate Solution is light-sensitive, avoid prolonged exposure to light. Dispense the TMB Substrate Solution within 15 minutes following the washing of the microtiter plate. In addition, avoid contact between TMB Substrate Solution and metal to prevent colour development. TMB is contaminated if it turns blue colour before use and should be discarded. TMB is toxic, avoid direct contact with hands.
6. Bacterial or fungal contamination of either samples or reagents or cross-contamination, between reagents may cause erroneous results.

## Samples Preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
2. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

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3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

## Assay Procedure

Prior to assay, allow reagents to stand at room temperature.

Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipette 25 $\mu$ L of Insulin standards, control and patient's sera into appropriate wells.
3. Add 100 $\mu$ L of Insulin Conjugate Reagent to all wells. Mix well, for 20 seconds.
4. Incubate for 60 minutes at room temperature (20-25 °C).
5. Remove liquid from all wells. Wash wells three times with 300  $\mu$ L of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 $\mu$ L of TMB substrate into all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50 $\mu$ L of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution

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## Calculation of Results

The standard curve is constructed as follows:

1. Check Insulin standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit.
2. To construct the standard curve, plot the absorbance for the insulin standards (vertical axis) versus the insulin standard concentrations in  $\mu\text{IU/mL}$  (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. Value above the highest point of the standard are retested after diluting with "0" standard.

Concentration( $\mu\text{IU/mL}$ )	OD(450nm)
0	0.007
5	0.113
25	0.526
50	0.914
100	1.397
300	2.225

## Expected Values

It is strongly recommended that each laboratory should determine its own normal and abnormal values. In a study conducted with apparently normal healthy adults, using the Insulin ELISA the following values are observed:  $< 25 \mu\text{IU/mL}$ .

## Limitations of the Test

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

## Declaration

1. The kit may not be suitable for special experimental samples where the validity of the experiment itself is uncertain, such as gene knockout experiments.
2. Certain natural or recombinant proteins, including prokaryotic and eukaryotic recombinant proteins, may not be detected because they do not match the detection antibody and capture antibody used in this product.
3. This kit is not compared with similar kits from other manufacturers or products with different methods to detect the same object, so inconsistent test results cannot be ruled out.

## Analysis of Common Problems and Causes of ELISA Experiment

### High background/Non-specific staining

Description of results	Possible reason	Recommendations and precautions
After termination, the whole plate results show a uniform yellow or light colour; or the Standard curve is linear but the background is too high	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed.
	ELISA plate was not washed sufficiently	Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated-Conjugate or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution.
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate.
	Colour development time is too long	Please strictly follow the steps of the manual.
	The wrong filter was used when the absorbance value was read	When TMB is used as the substrate, the absorbance should be read at 450 nm.



## No colour plate

Description of results	Possible reason	Recommendations and precautions
After the colour development step, all wells of the ELISA plate are colourless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when preparing or using
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the colour developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as $\text{NaN}_3$ , etc.), and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

## Light colour

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the colour of the sample is light	The sample uses $\text{NaN}_3$ preservative, which inhibits the reaction of the enzyme	Samples cannot use $\text{NaN}_3$
	The sample to be tested may The standard is normal, the colour of the sample is lightnot contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm.

Description of results	Possible reason	Recommendations and precautions
	Insufficient incubation time	Timer accurate timing
	Insufficient colour reaction	Usually 15-30minutes
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage.
	Distilled water quality problem	The prepared lotion must be tested to see if the pH value is neutral.

All wells, including Standard and Samples, are lighter in colour	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the colour developing agent.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as $\text{NaN}_3$ , etc.), confirm that the container for preparing the Washing Solution has been washed, and confirm that the purified water for preparing the Washing Solution meets the requirements and is not contaminated.
	The kit has expired or been improperly stored	Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination.
	Reagents and samples are not equilibrated before use	All reagents and samples should be equilibrated at room temperature for about 30 minutes.
	Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean.	To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.

Description of results	Possible reason	Recommendations and precautions
Poor repeatability	Incubation temperature constant temperature effect is not good	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells	When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.
	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints.
		Technical repetition of the same sample for 3 times, including more than 2 approximate values.
	Cross-contamination during sample addition	Try to avoid cross-contamination when adding samples

The colour of plate is chaotic and irregular	Cross-contamination from manual plate washing	When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination.
	Cross-contamination when clapping	Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination.

Description of results	Possible reason	Recommendations and precautions
The colour of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the colour of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual

For further details, contact us at

Origin Diagnostics and Research XIII/712 Origin Building, Clappana, Karunagappally, Kollam - 690525

[info@originlab.in](mailto:info@originlab.in) | +917736957333 | [www.originlab.in](http://www.originlab.in)