



# **Ultra Sensitive Mouse Insulin ELISA Kit, 2<sup>nd</sup> Gen Instructions**

For the quantitative determination of insulin in  
mouse serum, plasma, and cell culture media

**Catalog #62100  
Or #62105 (10 Kit Bundle)**

**96 Assays**

**For research use only. Not for use in diagnostic  
procedures.**

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**A. *Intended Use***

The Ultra Sensitive Mouse Insulin ELISA Kit, 2<sup>nd</sup> Generation (2<sup>nd</sup> Gen) is for the quantitative determination of insulin in mouse serum, plasma, and cell culture media. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

**B. *Introduction***

Insulin is the primary hormone produced in the  $\beta$  cells of the islets of Langerhans. It is known to play an important role in regulating glucose metabolism, i.e. the uptake of blood glucose to the liver and peripheral tissues. In addition, insulin plays an important role in other important physiological processes.

Recent increases in the incidence of diabetes and obesity have prompted intensive research on the effects of modulating and monitoring insulin levels and its production. As a result, the accurate measurement of insulin in experimental animals is important.

**C. *Principles of the Assay***

The Ultra Sensitive Mouse Insulin ELISA Kit, 2<sup>nd</sup> Gen is an ELISA sandwich assay for mouse insulin that uses only one intermediate wash step. The kit uses a pair of highly specific monoclonal antibodies for mouse insulin. One antibody is coated to the surface of the 96-well plate, and the second is labeled with HRP (horseradish peroxidase). To begin, samples and standards are added to each well along with HRP-labeled antibodies. The mouse insulin in the samples or standards binds to both the antibodies coated on

the well surface and to the HRP-labeled antibodies in solution. The mouse insulin gets 'sandwiched' between the two antibodies to form a complex on the surface. After incubation, the plate is washed to remove the excess HRP-labeled antibodies.

The amount of mouse insulin in each well is then determined by a colorimetric reaction. TMB (3,3',5,5'-tetramethylbenzidine) is added to each well. The HRP bound to the surface as part of the antibody-insulin-antibody complex reacts with the TMB resulting in a color change from clear to blue. The reaction is stopped, and the now yellow color is directly proportional to the amount of HRP present, which is proportional to the amount of mouse insulin present in the well. By comparing the magnitude of the color change relative to the known standards, the quantity of mouse insulin in the sample can be calculated.

#### ***D. Kit Storage***

1. Upon receipt of the Ultra Sensitive Mouse Insulin ELISA Kit, 2<sup>nd</sup> Gen, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

**E. Assay Materials****E.1. Materials provided****TABLE 1 Contents of the kit**

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8-well strips)	1 pack
STD 0-7	Standards (0, 0.09, 0.2, 0.4, 1, 2.5, 6 and 11 ng/mL)	8 x 1 mL
DIL	Assay Diluent	1 x 20 mL
CONJ	Antibody-HRP Conjugate (11X Concentrate)	1 x 0.8 mL
WASH	Wash Buffer (21X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL
	Adhesive covers for plate	2x, adhesive

**E.2. Materials to be supplied by user**

Micropipettes and disposable tips

Distilled or deionized water

Polypropylene microtubes

Volumetric flasks

Microplate reader (capable of reading  $A_{450}$  and  $A_{630}$  values)

**F. Assay Precautions**

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be

considered potentially hazardous. Avoid ingestion and contact with skin and eyes.

2. Some assay components may contain human or animal sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Some reagents are light sensitive and should be protected from sunlight.

### ***G. Maximizing Kit Performance***

1. Given the small sample volumes required (5  $\mu\text{L}$ ), pipetting should be done as carefully as possible. A high quality 10  $\mu\text{L}$ , or better, precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures to ensure consistent time intervals between wells for each process.
5. Do not mix reagents that have different lot numbers.
6. Do not leave the substrate open to ambient light until just prior to use.

## **H. Preparation of Mouse Plasma and Serum**

**Plasma:** Collect blood in a tube containing an anticoagulant such as heparin (final concentration: 1 unit/mL) or EDTA (final concentration: 0.1%), and centrifuge at 4°C for 20 min at 2,000 x g.

**Serum:** Collect blood, allow to clot, and centrifuge at 4°C for 20 min at 2,000 x g.

**Note:** *Samples can be stored at 2-8°C for 24 hours prior to analysis in this assay. For longer periods, storage at <-20°C is recommended. Avoid repeated freeze/thaw cycles. Avoid hemolysis during preparation. Do not use turbid serum or plasma samples. Turbid serum or plasma should be centrifuged prior to use. Samples which need to be diluted must be diluted with the Assay Diluent (marked "DIL").*

## **I. Assay Procedure**

### **I.1. Preparation of reagents**

1. **MIC:** Antibody-coated microplate (12 x 8-well strips)  
Provided as ready to use.
2. **STD:** Standards 0 - 7  
Provided in liquid, ready to use format ranging from 0 ng/mL to 11 ng/mL. Standards are provided in the following concentrations: 0, 0.09, 0.2, 0.4, 1, 2.5, 6, and 11 ng/mL.
3. **DIL:** Assay Diluent  
Provided as ready to use.
4. **CONJ:** Antibody-HRP Conjugate (11X Concentrate)  
The Antibody-HRP Conjugate has to be diluted 1:11 with assay diluent just prior to use. Prepare only enough 1X conjugate as needed. For a whole plate, add 700 µL of

concentrate to 7 mL of assay diluent or for 6 strips add 350  $\mu$ L to 3.5 mL. Gently mix to ensure homogeneity. The working HRP solution should be used shortly after it is prepared. Protect from light.

5. **WASH:** Wash Buffer (21X Concentrate)

The Wash Buffer has to be diluted 1:21 with distilled or deionized water prior to use. Crystal formation in the concentrate is not uncommon when stored at low temperatures. Accordingly, the wash buffer concentrate should be allowed to sit at room temperature (or gently warmed) for at least 30 minutes (and then mixed gently to allow the crystals to fully dissolve). Once any crystals present have dissolved, prepare enough 1X wash buffer as needed. To prepare wash buffer for the full plate, 50 mL of Wash Buffer must be diluted with 1,000 mL of distilled or deionized water. The prepared wash buffer is stable for at least 5 days at 2-8°C.

**Note:** *It is not necessary to have the undiluted wash buffer ready prior to starting the assay as it is only needed later in the procedure. We recommend you remove the concentrate from the fridge when you start the assay to provide adequate time for the concentrate to warm to room temperature prior to diluting.*

6. **SUB:** Substrate Solution

Provided as ready to use. Protect from light.

7. **STOP:** Stop Solution

Provided as ready to use.



## 1.2. Assay procedure

Prior to running the assay or opening any containers, all reagents should be brought to room temperature (20–22 °C) for at least 30 minutes. Mix the reagents thoroughly by gentle agitation or swirling prior to use, and then return the reagents to 2–8°C after use. All samples should be mixed or vortexed to ensure a homogenous composition. Using the plate diagram on page 13, plan locations for standards and samples, and if only a partial plate is needed, remove the desired number of strips. Unused strips should be returned and stored in the resealed pouch with the desiccant at 2–8°C for future use.

1. In each well, add 50 µL of Assay Diluent (DIL).
2. In the appropriate well, add 5 µL of sample or standard (STD).
3. In each well, add 50 µL of 1X antibody-HRP conjugate.  
**Note:** *Conjugate must be added to all wells, including to the zero standard (0 ng/mL).*
4. Cover the wells with the adhesive plate seal and carefully shake the plate by hand for 15 seconds to mix. Avoid spillage.
5. Incubate the sealed plate for 2.5 hours at room temperature without shaking.
6. Remove the adhesive seal and aspirate (or dump out) well contents. Wash a total of six times by dispensing and then aspirating 300 µL of wash buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of Substrate Solution (SUB) in each well.
8. Cover the wells with the adhesive plate seal and incubate the plate for 30 mins in the dark at room

temperature. Do not use aluminum foil to cover the wells.

9. Add 100  $\mu\text{L}$  of Stop Solution (STOP) to each well to stop the reaction.
10. Visually inspect the wells to ensure there are no bubbles that will interfere with the assay. Burst any bubbles with a clean pipette tip.
11. Measure the absorbance of each well within 15 minutes at 450nm using a plate reader. If wavelength correction is available on the plate reader, set to 630 nm. If wavelength correction is not available, subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for any optical imperfections in the plate that might influence absorbance readings.

### **I.3. Determining the mouse insulin concentration**

1. Calculate the average absorbance reading for each set of samples or standards.
2. Construct the mouse insulin calibration curve by plotting the average absorbance value for each standard on the Y axis versus the corresponding mouse insulin concentration on the X axis. Plot the zero standard (0 ng/mL) as part of the curve.

**Note:** *A calibration curve should be plotted every time the assay is performed.*

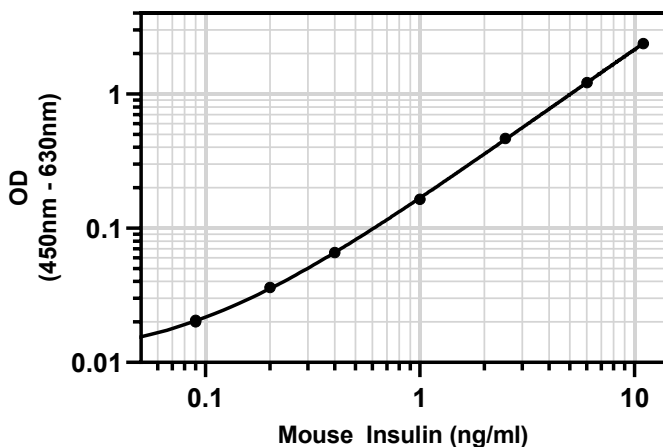
To prepare the most accurate curve, use computer software and a 4 or 5 parameter logistic (4PL or 5 PL) curve fit.

If a 4PL or 5PL curve cannot be used, a linear regression fit is recommended as an alternative. The data may be linearized by plotting the log of the concentrations on the X axis versus vs. the log of the average ODs on the Y axis and then using regression analysis to determine the best linear fit. *This method will produce an adequate but less precise fit of the data.*

3. Mouse insulin concentrations in the samples are interpolated using the calibration curve. For diluted samples, the values obtained must be multiplied by the dilution factor to obtain the final insulin concentration. The insulin concentration is expressed in ng/mL.

**Note:** *Samples with high mouse insulin concentrations (ie. fall above the range of the assay) should be diluted with the Assay Diluent and rerun.*

**Figure 1: A typical standard curve**



***J. Performance characteristics***

**J.1. Assay range and analytical sensitivity**

The Mouse Insulin ELISA Kit has an assay range from 0.09 – 11 ng/mL. The analytical sensitivity of the assay is  $\leq 0.05$  ng/mL using a 5  $\mu$ L sample.

**J.2. Precision**

The assay has a within-run and total precision of  $CV \leq 10\%$ .

***Warranty***

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**Notes:**

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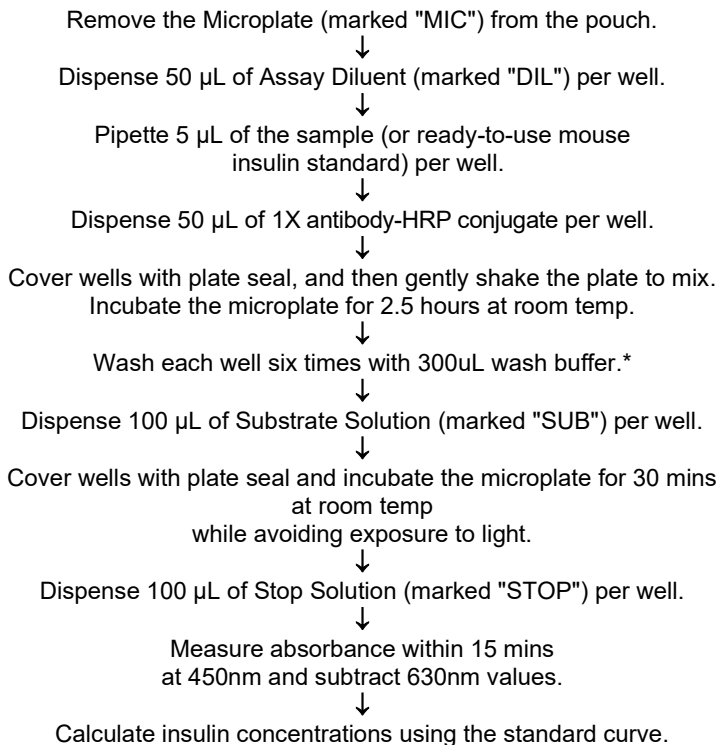
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**Plate Map Record:** Use this to record sample locations.

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	2										
	3										
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### **Summary**

#### **Ultra Sensitive Mouse Insulin ELISA Kit, 2nd Gen**



\* For optimal results, tap the empty plate firmly on a paper towel after each wash step to ensure all buffer is removed.

#### **HIGHLIGHTS**

**Wide dynamic range:** 0.09 – 11 ng/mL using only a 5  $\mu$ L sample

**Easy-to-use procedure:** Only one wash step

**High sensitivity:**  $\leq$  0.05 ng/mL using only a 5  $\mu$ L sample

**Convenient:** Ready-to-use liquid standards