# Mammalian Insulin ELISA Instructions

## Cat:EH0038

## Content

| | CAT | Volume |
|---|---|---|
| ❶ CP (Coated Plate) | EH0038CP | 96 well |
| ❷ S (Standard) | EH0038S,S1~S7,S0 | 9 vial |
| ❸ DA (Detect Antibody) | EH0038DA | 6 ml/bottle |
| ❹ SH (Streptavidin-HRP) | ESH01 | 12 ml/bottle |
| ❺ AB (Assay Buffer 1×) | EAB01 | 12 ml/bottle |
| ❻ TS (TMB Substrate) | ETS01 | 12 ml/bottle |
| ❼ SS (Stop Solution) | ESS01 | 12 ml/bottle |
| ❽ WB (Wash Buffer 10×) | EWB01 | 50 ml/bottle |
| ❾ SF (Sealer Film) | ESF01 | 6 pieces |

**NOTE:** After the kit is opened, the stabilization period of each content is 30 days, so please use the kit within 30 days after opening.

## REAGENT PREPARATION

### Washing Buffer (1×) Preparation

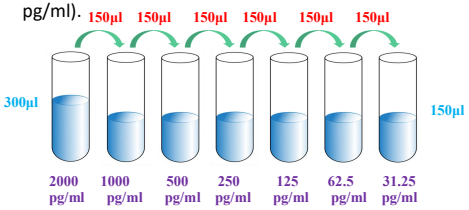
Pour entire contents (50 ml) of the **Washing Buffer Concentrate** (10×) into a clean 500 ml graduated cylinder. Bring to final volume of 500 ml with glass-distilled or deionized water. Transfer to a clean wash bottle and store at 2 to 25℃.

### Standard Curve Preparation:

S1 to S7 and S0 is ready to use for serum and plasma.

Other sample type, prepare the standard curve with whatever buffer (SPB, Sample Prepared Buffer) is used to prepare the sample, such as cell culture supernatant, tissue grinding liquid, cell lysate, etc. Urine sample use AB (Assay Buffer) prepare standard curve.

The Mammalian Insulin Standard EH0038S 20000 pg/ml 30 µl + 270 µl SPB serves as the high standard (2000 pg/ml). Pipette 150 µl of SPB into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. SPB serves as the zero standard (0 pg/ml).




## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use.**

❶ Prepare all reagents and working standards as directed in the previous sections.

❷ Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.

❸ Add 50 µl of **AB** (Assay Buffer) to each well.

❹ Add 50 µl or 20 µl of **Standard or sample** per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.

❺ Add 50 µl of **DA** (Detect Antibody) to each well.

❻ Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 1 hour on a microplate **shaker** set at 500 rpm.

❼ Aspirate each well and **wash**, repeating the process four times. Wash by filling each well with WB (Washing Buffer 300 µl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining WB (Washing Buffer) by aspirating or decanting. Invert the plate and **blot** it against clean paper towels.

❽ Add 100 µl of **SH** (Streptavidin-HRP) to each well.

❾ Cover with a new **SF** (Sealer Film). Incubate at room temperature (18 to 25℃) for 30 min on a microplate **shaker** set at 500 rpm.

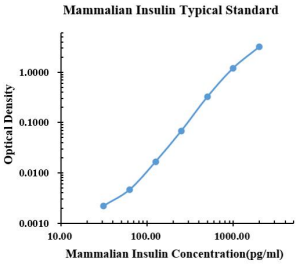
❿ Repeat aspiration/**wash** as in step 7.

⓫ Add 100 µl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.

⓬ Add 100 µl of **SS** (Stop Solution) to each well.

⓭ Determine the optical density within 30 minutes, using microplate **reader** set to 450 nm

## TYPICAL DATA




| pg/ml | O.D. | | Average | Corrected |
|---|---|---|---|---|
| 0.00 | 0.0081 | 0.0076 | 0.0079 | |
| 31.25 | 0.0100 | 0.0101 | 0.0101 | 0.0022 |
| 62.50 | 0.0122 | 0.0127 | 0.0125 | 0.0046 |
| 125.00 | 0.0245 | 0.0242 | 0.0244 | 0.0165 |
| 250.00 | 0.0767 | 0.0754 | 0.0761 | 0.0682 |
| 500.00 | 0.3493 | 0.3107 | 0.3300 | 0.3222 |
| 1000.00 | 1.2640 | 1.1620 | 1.2130 | 1.2052 |
| 2000.00 | 3.2150 | 3.1260 | 3.1705 | 3.1627 |

## SENSITIVITY

The minimum detectable dose (MDD) of Mammalian Insulin is typically less than 20.89 pg/ml (50 µl of sample volume) or 28.28 pg/ml (20 µl of sample volume).

The MDD was determined by adding two standard deviations to the mean optical density value of ten zero standard replicates and calculating the corresponding concentration.

## PRECISION

**Intra-assay Precision (Precision within an assay)** Three samples of known concentration were tested twenty-two times on one plate to assess intra-assay precision.

**Inter-assay Precision (Precision between assays)**

| | Intra-assay Precision | | | Inter-assay Precision | | |
|---|---|---|---|---|---|---|
| Sample Number | S1 | S2 | S3 | S1 | S2 | S3 |
| | 22 | 22 | 22 | 6 | 6 | 6 |
| Average（pg/ml） | 581.0 | 2316.1 | 7446.9 | 579.8 | 2277.0 | 7736.2 |
| Standard Deviation | 12.8 | 34.2 | 243.7 | 14.4 | 34.5 | 230.5 |
| Coefficient of Variation（%） | 2.2 | 1.5 | 3.3 | 2.5 | 1.5 | 3.0 |

## RECOVERY

The spike recovery was evaluated by spiking 3 levels of Mammalian Insulin into health serum sample. The un-spiked serum was used as blank in this experiment.

The recovery ranged from 80% to 120% with an overall mean recovery of 110%.

## LINEARITY

To assess the linearity of the assay, five samples were spiked with high concentration of Mammalian Insulin in serum and diluted with Sample Diluent to produce samples with values within the dynamic range of the assay.

The linearity ranged from 81% to 119% with an overall mean recovery of 109%.

## SAMPLE VALUES

Serum/Plasma – Thirty samples from apparently healthy mice/healthy volunteers/healthy rats were evaluated for the presence of Mammalian Insulin in this assay. No medical histories were available for the donors.

| Sample Matrix | Sample Evaluated | Range (pg/ml) | Detectable % | Mean of Detectable (pg/ml) |
|---|---|---|---|---|
| Mouse Serum | 30 | n.d-289.05 | 90 | 41.29 |
| Rat Serum | 30 | 13.76-924.50 | 100 | 106.12 |
| Human Serum | 30 | 132.03-2758.63 | 100 | 1027.08 |

n.d. = non-detectable. Samples measured below the sensitivity are considered to be non-detectable.