MSD® 384-Well MULTI-Array® MOUSE/RAT INSULIN ASSAY

The following assay protocol has been optimized for analysis of mouse or rat Insulin in serum and plasma samples.

MSD Materials

	Storage
❑ Read Buffer T (with surfactant), 4X	RT
❑ Blocker A Kit	2-8°C
❑ Antibody Diluent	2-8°C
❑ MULTI-ARRAY, 384-well Custom plates	2-8ºC
❑ SULFO-TAG™ Insulin Detection Antibody (100X)	2-8ºC
❑ Metabolic Assay Diluent	-20ºC
❑ Mouse/Rat Insulin Calibrator	-20ºC

Other Materials & Equipment (not supplied)

- ❑ Deionized water for diluting Read Buffer
- ❑ Various microcentrifuge tubes for making serial dilutions (if desired)
- ❑ Automated plate washer, Multidrop®, or other efficient multi-channel pipetting equipment for washing 384 well plates
- ❑ Appropriate liquid handling equipment for desired throughput that must accurately dispense 5 – 100 µL into a 384-well micro plate
- ❑ PBS Buffer, Sigma Dulbecco's PBS (catalog # D5652) is recommended
- ❑ Adhesive Plate Seals

Protocol at a Glance

The following protocol describes the most conservative approach toward achieving highly sensitive results using MSD technology to measure mouse or rat Insulin in serum and plasma samples. The protocol can be completed in approximately 3 to 3½ hours if each reagent is prepared during the preceding blocking or incubation step. Alternatively, the plates can be blocked overnight at 4°C to decrease the remaining experimental time to 2 hours.

Step 1. Block with Blocker A solution for 1 hour at room temperature or overnight at 4°C, wash with PBS.
Step 2. Add Metabolic Assay Diluent containing Detection Antibody.
Add mouse or rat serum/plasma samples or Calibrator.
Incubate with shaking for 2 hours, wash with PBS.
Step 3. Add Read Buffer.
Read Plate on Sector Imager.
Step 4. Analyze data.

Notes:

Read the entire detailed instructions before beginning work.

The Blocking Step can be omitted without significant loss in assay performance.

Detailed Instructions

Prepare Blocker A solution:
Follow instructions included with the Blocker A Kit.

Solutions containing Blocker A should be kept at 4ºC and discarded after 14 days.

Dilute Read Buffer:
Dilute 4X Read Buffer T to 1X with deionized water. Approximately 20 mL of 1X Read Buffer is required per plate.

Diluted Read Buffer may be kept in a tightly sealed container at room temperature for later use.

Prepare the Mouse/Rat Insulin Calibrators:

a) Dilute Insulin stock 100-fold by adding 5 µL of 0.14 mg/mL Insulin to 495 µL Antibody Diluent. The resulting concentration of diluted stock is 1.4 µg/mL.
b) Prepare highest Calibrator by adding 5 µL of 1.4 µg/mL Insulin to 135 µL of Antibody Diluent.
c) Prepare the next Calibrator by transferring 40 µL of the highest Calibrator to 80 µL of Antibody Diluent.
d) Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.

e) This yields the following Calibrator concentrations:

Calibrator	Insulin (pg/mL)
Cal 7	50,000
Cal 6	16,667
Cal 5	5,555
Cal 4	1,852
Cal 3	617
Cal 2	206
Cal 1	68
Cal 0	0

f) Use Antibody Diluent for Cal 0. These Calibrators will be sufficient to run an 8-point calibration curve in duplicate for 5 plates, allowing extra "dead" volume for pipetting technique. Do not store diluted Calibrators for more than 4 hours.

Notes:

Prepare Sample:

The assay format requires 5 µL of serum or plasma samples per well. An adequate volume of each sample should be prepared depending upon desired number of replicates.

Prepare Insulin Detection Antibody:

a) Thaw Metabolic Assay Diluent.
b) In 15 mL tube, combine 80 µL of 100X Detection Antibody Solution and 7920 µL of Metabolic Assay Diluent. Mix thoroughly. This will yield 8 mL of diluted Insulin detection antibody mixture at the working concentration, with sufficient volume for an entire plate.

STEP 1

Block Plate:

a) Add 40 µL/well of Blocker A Solution.
b) Incubate at room temperature for 1 hour; mixing is not required.
c) Wash plate 3 times with PBS before proceeding to the next step.

Plates may also be blocked overnight at 4ºC.

Always pipette sample at the bottom corner of the well.

STEP 2

Start the Incubation:

a) Add 15 µL/well of diluted Insulin Detection Antibody while plate is wet.
b) Immediately following antibody addition, add 5 µL/well Insulin Calibrators and 5 µL/well samples. It is recommended that both Calibrators and samples be assayed in duplicate. Cover plate with an adhesive plate sealer and incubate on a plate shaker (low-medium setting) for 2 hours.
d) Wash plate with PBS.

STEP 3

Read Plate:

a) Add 35 µL/well of 1X Read Buffer T.
b) Read plate immediately (within a few minutes of filling it with Read Buffer) on SECTOR™ Imager.
c) Discard plate after read step.

STEP 4

Analyze Data:

Always use curves obtained from the Calibrator dilution series to calculate Insulin levels in the samples. A 4-parameter logistic fit (4PL fit) should be used, and can provide assistance with data analysis as needed.

Notes:

Note that bubbles in the Read Buffer will interfere with reliable imaging of the plate if carried into the wells.

Plates can be imaged immediately following the addition of read buffer. Most biological interactions tolerate incubation in Read Buffer however each unique assay should be tested for stability in read buffer before being left to sit for extended periods.