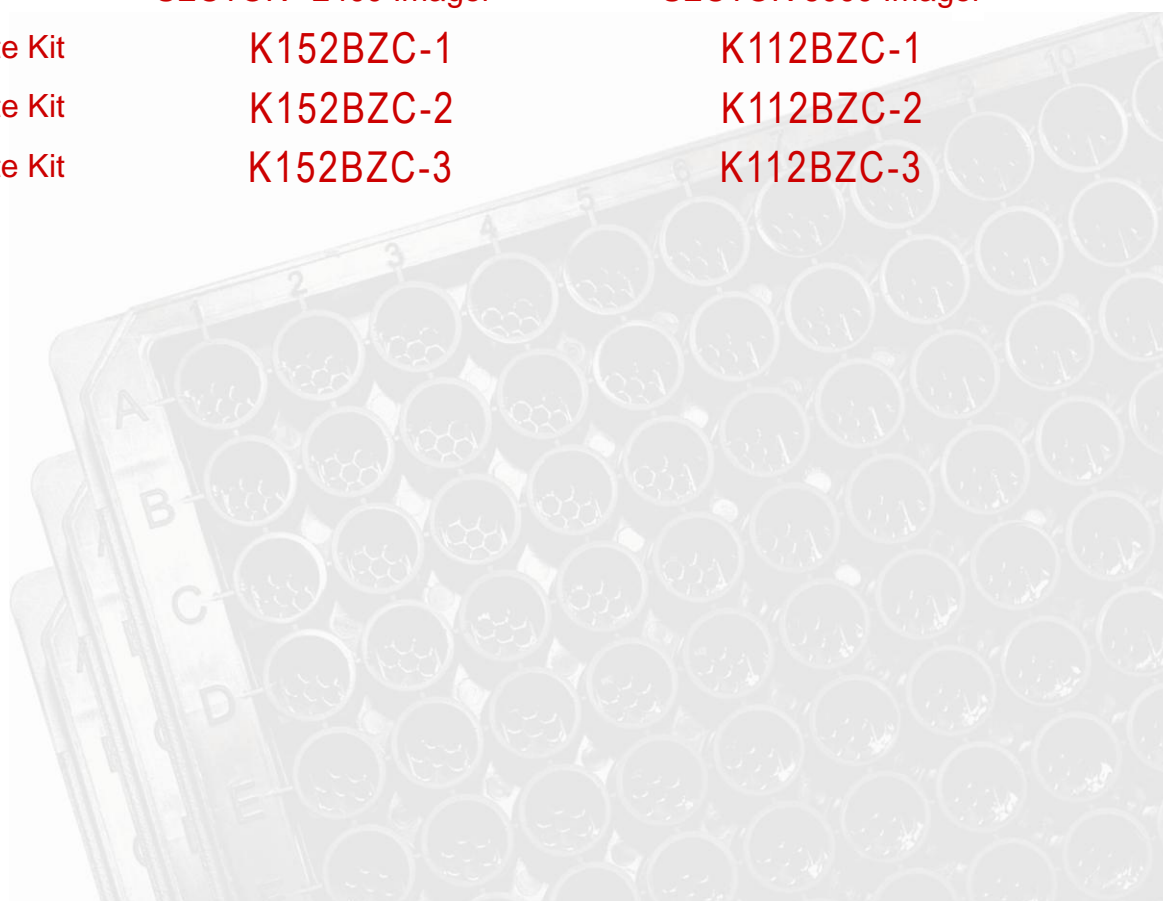


Meso Scale Discovery[®]

MULTI-ARRAY[®] Assay System

Mouse/Rat Insulin Assay Kit

	SECTOR [®] 2400 Imager	SECTOR 6000 Imager
1-Plate Kit	K152BZC-1	K112BZC-1
5-Plate Kit	K152BZC-2	K112BZC-2
20-Plate Kit	K152BZC-3	K112BZC-3



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MSD Metabolic Assays

Mouse/Rat Insulin Assay Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

Meso Scale Discovery

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Ordering Information

ordering information

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Introduction

introduction

Insulin is a 51-residue peptide hormone that is produced in the pancreas by β -cells of the islets of Langerhans. Insulin is involved in the regulation of carbohydrate, fat and protein metabolism. Lowered levels of insulin cause liver cells to convert glycogen back to glucose and secrete it into the blood. Insulin also has an effect on small vessel muscle tone, storage and release of (fat) triglycerides and cellular uptake of amino acids and electrolytes. Type 1 diabetes results when the β -cells are destroyed and no longer producing insulin resulting in high glucose levels in the blood. Patients with type 1 diabetes depend on exogenous insulin for their survival because of an absolute deficiency of the hormone; patients with type 2 diabetes have either relatively low insulin production or insulin resistance or both.

Principle of the Assay

principle of the assay

MSD[®] metabolic assays provide rapid and convenient methods for measuring the levels of protein targets within single small-volume samples. The assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or “spot”) per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Mouse/Rat Insulin Assay detects insulin in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with insulin antibody. The user adds the sample and a solution containing the labeled detection antibody—anti-insulin labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—over the course of one or more incubation periods. Insulin in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound analyte completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of insulin present in the sample.

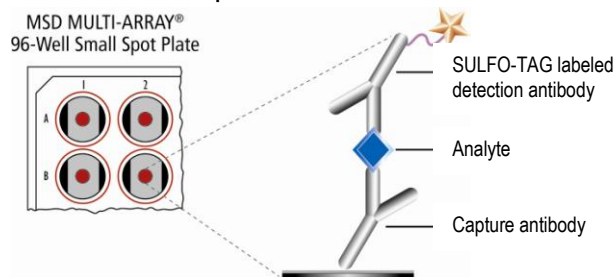


Figure 1. Sandwich immunoassay on MSD platform



Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K152BZC-1 K112BZC-1	K152BZC-2 K112BZC-2	K152BZC-3 K112BZC-3
MULTI-ARRAY 96-well Mouse/Rat Insulin Plate(s) L452BZA-1 (K152BZC) L412BZA-1 (K112BZC)	2-8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-m/r Insulin Antibody ¹ (100X)	2-8°C	1 vial (60 µL)	1 vial (300 µL)	4 vials (300 µL ea)
Insulin Calibrator 5 µg/mL	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	20 vials (15 µL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	4 bottles (250 mL ea)
Diluent 100 R50AA-4 (50 mL) R50AA-2 (200 mL)	2-8°C	1 bottle (50 mL)	1 bottle (50 mL)	1 bottle (200 mL ea)
Diluent 17 R50KA-4 (6 mL) R50KA-3 (30 mL)	≤-10°C	1 bottle (6 mL)	1 bottle (30 mL)	4 bottles (30 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL) R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	1 bottle (200 mL)



Required Materials and Equipment - not supplied

required materials and equipment — not supplied

- Deionized water for diluting concentrated buffers
- 50 mL tubes for reagent preparation
- 15 mL tubes for reagent preparation
- Microcentrifuge tubes for preparing serial dilutions
- Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- Appropriate liquid handling equipment for desired throughput, capable of dispensing 25 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker



Safety

safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

¹ Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

VI Reagent Preparation

reagent preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

Important: Upon first thaw, separate Diluent 17 into aliquots appropriate to the size of your assay needs. This diluent can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Calibrator and Control Solutions

Calibrator for the Mouse/Rat Insulin Assay is supplied at 5 µg/mL. For the assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	Insulin conc. (pg/mL)	Dilution Factor
Stock Cal. Vial	5000000	
STD-01	50000	100
STD-02	16667	3
STD-03	5556	3
STD-04	1852	3
STD-05	617	3
STD-06	206	3
STD-07	69	3
STD-08	0	n/a

To prepare this 8-point standard curve:

- 1) Prepare the highest Calibrator by transferring 10 µL of the Calibrator stock vial to 990 µL of Diluent 100.
- 2) Prepare the next Calibrator by transferring 100 µL of the diluted Calibrator to 200 µL of Diluent 100. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) The recommended 8th Standard is Diluent 100 (i.e. zero Calibrator).
- 4) Diluted Calibrators should be kept on ice prior to addition to the plate.

Note: The standard curve can be modified as necessary to meet specific assay requirements.

Preparation of Serum and Plasma Samples

The assay format requires 10 µL of sample per well. An adequate volume of each sample should be prepared depending upon desired number of replicates.

Prepare Detection Antibody Solution

The Detection Antibody is provided at 100X stock solution. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute a 50 µL aliquot of the stock Detection Antibody into 4.95 mL of Diluent 17.

Prepare Read Buffer

The Read Buffer should be diluted 4-fold in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of 4X Read Buffer T to 15 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibody for the analyte shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

assay protocol

1. **Addition of Blocker A Solution:** Dispense 150 μ L of Blocker A Solution into each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
2. **Wash and Addition of the Detection Antibody Solution followed by Sample or Calibrator:** Wash the plate 3 times with PBS-T. Dispense 40 μ L of 1X Detection Antibody Solution into each well of the MSD plate. Immediately add 10 μ L of sample or Calibrator into the appropriate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
3. **Wash and Read:** Wash the plate 3 times with PBS-T. Add 150 μ L of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

Notes

Shaking a 96-well MSD MULTI-ARRAY plate typically accelerates capture at the working electrode.

Bubbles in the fluid will interfere with reliable reading of MULTI-ARRAY plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

VIII Analysis of Results

analysis of results

The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantification in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH[®] analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

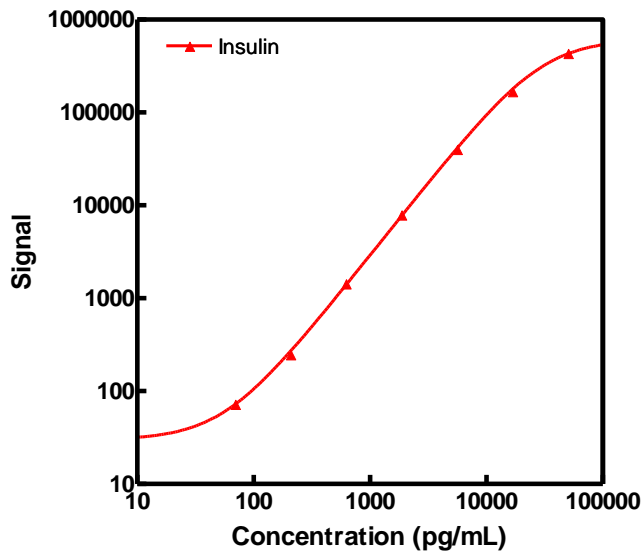
IX

Typical Standard Curve

typical standard curve

The MSD Mouse/Rat Insulin Assay is designed for use with mouse and rat serum and plasma samples.

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary. A standard curve should be run for each set of samples and on each plate for the best quantification of unknown samples.



Insulin		
Conc. (pg/mL)	Average Signal	%CV
0	42	9.7
69	73	8.9
206	249	3.2
617	1441	4.3
1852	7968	8.9
5556	40493	11.3
16667	168989	4.2
50000	436781	1.6

X

Sensitivity

sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero Calibrator. The value below represents the average LLOD over multiple kit lots.

Insulin	
LLOD (pg/mL)	35

XI Spike Recovery

spike recovery

Serum, EDTA plasma, and heparin plasma were spiked with the Calibrators at multiple values throughout the range of the assay. Measured analyte represents average spike recovery in pooled mouse serum and plasma samples.

$$\% \text{ Recovery} = \text{measured} / \text{expected} \times 100$$

	Spike Conc. (pg/mL)	% Recovery
Spiked Serum	500	89
	2500	85
	5000	81
Spiked EDTA Plasma	500	96
	2500	88
	5000	87
Spiked Heparin Plasma	500	95
	2500	92
	5000	88

XII Linearity

linearity

Linearity was measured by spiking Calibrator levels in pooled mouse plasma followed by subsequent dilution.

Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).

$$\% \text{ Recovery} = \text{measured} \times \text{dilution factor} / \text{expected} \times 100$$

	Fold Dilution	% Recovery
Serum	2	96
	4	107
	8	98
EDTA Plasma	2	103
	4	109
	8	103
Heparin Plasma	2	110
	4	110
	8	104

XIII Assay Components

assay components

Calibrator source: Recombinant human insulin

The insulin Calibrator has been anchored and referenced to international standards. The table below summarizes the reference information.

Analyte	WHO Standard Reference Number	WHO Standard Units / μg	MSD Calibrator $1\mu\text{g} = \text{WHO Units}$	WHO Units
Insulin	66/304	0.023	0.023	IU

Capture Antibody	
Analyte	Mouse/Rat insulin
Source	Mouse monoclonal
Isoforms Recognized	Does not react with human proinsulin, rat or human C-peptide
Species cross-reactivity	Human, mouse, rat, porcine, bovine
Detection Antibody	
Analyte	Mouse/Rat insulin
Source	Mouse monoclonal
Isoforms Recognized	Does not react with human proinsulin, rat or human C-peptide
Species cross-reactivity	Human, mouse, rat, porcine, bovine

XIV References

references

1. Bristow AF, Das RE, Bangham DR. World Health Organization International Standards for highly purified human, porcine and bovine insulins. *J Biol Stand.* 1988 Jul;16(3):165-78.
2. Golla R, Seethala R. A sensitive, robust high-throughput electrochemiluminescence assay for rat insulin. *J Biomol Screen.* 2004 Feb;9(1):62-70
3. Plum L, Belgardt BF, Brüning JC. Central insulin action in energy and glucose homeostasis. *J Clin Invest.* 2006 Jul;116(7):1761-6
4. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature.* 2001. Dec 13;414(6865):799-806

Summary Protocol

MSD 96-well MULTI-ARRAY Mouse/Rat Insulin Assay Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Mouse/Rat Insulin Assay.

Step 1 : Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the Calibrator stock on ice.

Prepare Blocker A Solution.

Prepare serum or plasma samples.

Prepare an 8-point standard curve using supplied Calibrator:

- The Calibrator should be diluted in Diluent 100.
- Dilute the stock Calibrator 1:100 in Diluent 100 then perform a series of 3-fold dilution steps and a no Calibrator blank.
- Diluted Calibrators should be kept on ice until use.

Note: *The standard curve can be modified as necessary to meet specific assay requirements.*

Prepare Detection Antibody Solution by diluting the 100X Anti-m/r Insulin Antibody to 1X in a final volume of 3.0 mL of Diluent 17 per plate.

Prepare 20 mL of 1X Read Buffer T by diluting 4X Read Buffer T with deionized water.

Step 2 : Add Blocker A Solution

Dispense 150 μ L/well Blocker A Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 3 : Wash and Add Detection Antibody Solution Followed by Sample or Calibrator

Wash plate 3 times with PBS-T.

Dispense 40 μ L/well 1X Detection Antibody Solution.

Immediately, Dispense 10 μ L/well Calibrator or Sample.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4 : Wash and Read Plate

Wash plate 3 times with PBS-T.

Dispense 150 μ L/well 1X Read Buffer T.

Analyze plate on SECTOR instrument.

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