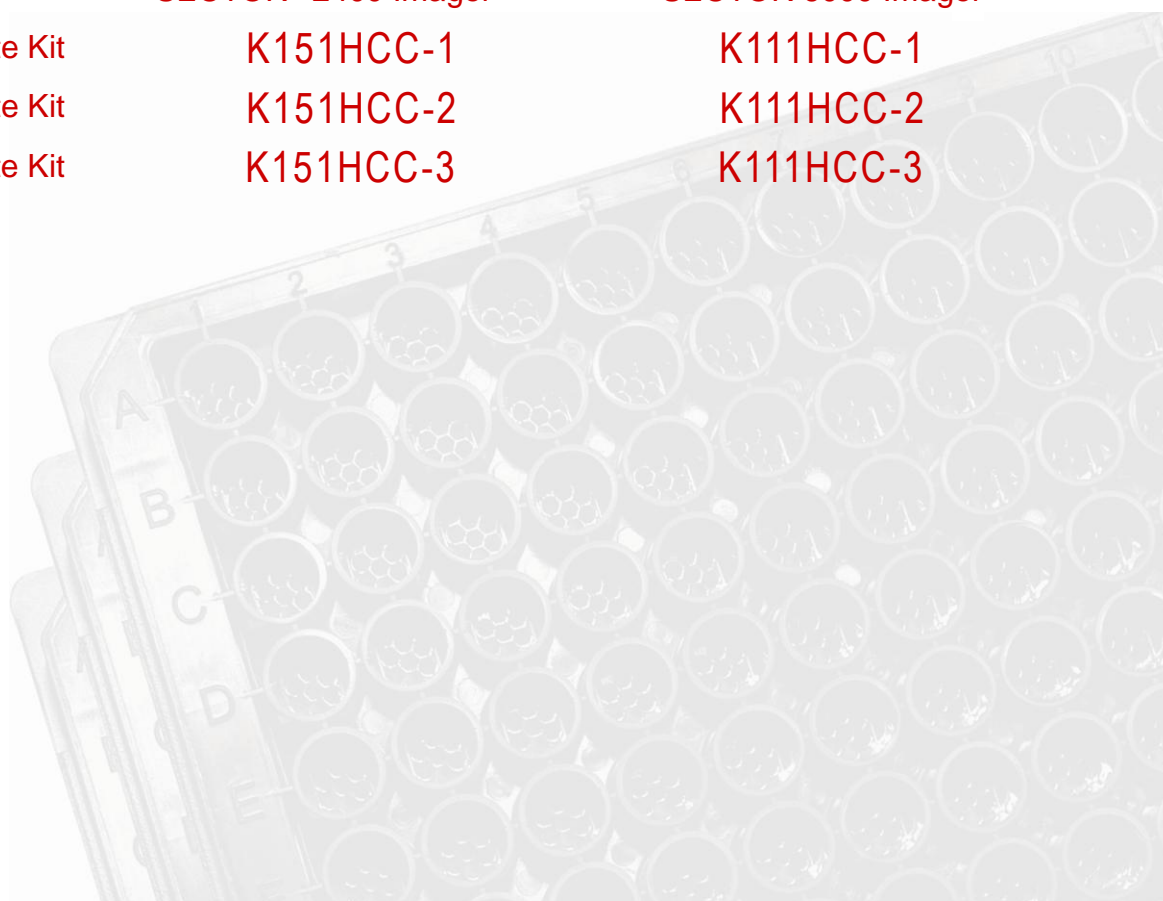


# Meso Scale Discovery<sup>®</sup>

## MULTI-ARRAY<sup>®</sup> Assay System

### Human Glucagon Assay Kit

	SECTOR <sup>®</sup> 2400 Imager	SECTOR 6000 Imager
1-Plate Kit	K151HCC-1	K111HCC-1
5-Plate Kit	K151HCC-2	K111HCC-2
20-Plate Kit	K151HCC-3	K111HCC-3



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# MSD Metabolic Assays

## Human Glucagon Assay Kit

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

**FOR RESEARCH USE ONLY.**

**NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

### Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.

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# Table of Contents

table of contents

I. Introduction .....	4
II. Principle of the Assay .....	4
III. Reagents Supplied.....	5
IV. Required Material and Equipment – not supplied .....	5
V. Safety.....	6
VI. Reagent Preparation.....	6
VII. Assay Protocol.....	8
VIII. Analysis of Results.....	8
IX. Typical Standard Curve .....	9
X. Sensitivity.....	9
XI. Spike Recovery.....	10
XII. Linearity .....	10
XIII. Assay Components.....	11
XIV. References .....	11
Summary Protocol .....	13
Plate Diagrams .....	15

## Ordering Information

ordering information

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# Introduction

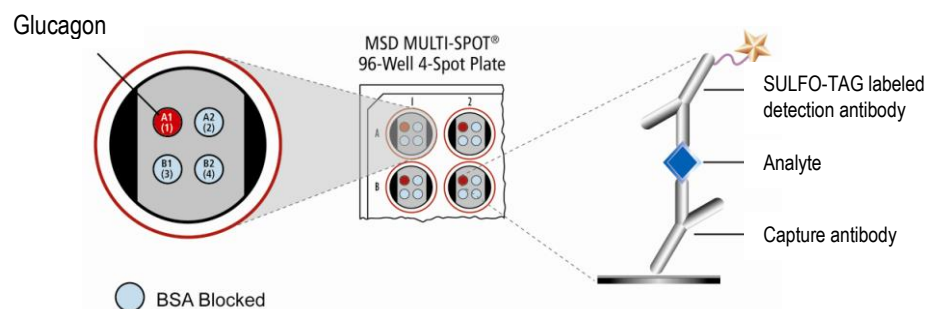
introduction

**Glucagon** is a 29-residue polypeptide hormone that is produced in the pancreas by the  $\alpha$ -cells of the islets of Langerhans. Glucagon is involved in maintaining normal levels of glucose in the blood by acting on liver glycogen, converting it to glucose. Glucagon is a stimulator of hepatic glycogenolysis, gluconeogenesis, and ketogenesis which are antagonistic effects to those of insulin action, resulting in increased blood glucose levels. Glucagon receptors have been found in liver, kidney, intestinal smooth muscle, brain and adipose tissue.

## Principle of the Assay

principle of the assay

MSD<sup>®</sup> 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 Human Glucagon Assay detects glucagon in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with glucagon antibody. The user adds the sample and a solution containing the labeled detection antibody—anti-glucagon labeled with an electrochemiluminescent compound, MSD SULFO-TAG<sup>™</sup> label—over the course of one or more incubation periods. Glucagon 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 glucagon present in the sample.



**Figure 1.** Sandwich immunoassay on MSD platform. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding to that spot. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

# Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K151HCC-1 K111HCC-1	K151HCC-2 K111HCC-2	K151HCC-3 K111HCC-3
MULTI-SPOT 96-well Glucagon Plate(s) N450HCA-1 (K151HCC) N410HCA-1 (K111HCC)	2-8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-Glucagon Antibody <sup>1</sup> (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
Glucagon Calibrator 1 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) R93AA-1 (1 L)	RT	1 bottle (250 mL)	1 bottle (250 mL)	4 bottles (250 mL ea)
Aprotinin (200,000 KIU/mL)	2-8°C	1 vial (50 µL)	1 vial (250 µL)	4 vials (250 µL ea)
Blocker D-B (10%)	≤-10°C	1 vial (1.2 mL)	2 vials (1.2 mL ea)	8 vials (1.2 mL ea)
Blocker E (100X)	≤-10°C	1 vial (0.09 mL)	1 vial (0.45 mL)	4 vials (0.45 mL ea)
Diluent 17 R50KA-3 (30 mL)	≤-10°C	1 bottle (30 mL)	2 bottles (30 mL ea)	6 bottles (30 mL ea)
Diluent 12 R50JA-4 (10 mL) R50JA-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	2 bottles (50 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 10 to 150 µL into a 96-well microtiter plate
- Plate washing equipment: automated plate washer or multichannel pipette
- Adhesive plate seals
- Microtiter plate shaker

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

# V Safety

s a f e t y

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.

# VI Reagent Preparation

## r e a g e n t   p r e p a r a t i o n

Bring all reagents to room temperature and thaw the Calibrator stock on ice. Blockers D-B and E can tolerate up to 5 freeze-thaw cycles. Alternatively, an aliquot of each blocker can be stored at 2-8°C for up to 1 month.

**Important:** Upon first thaw, separate Diluent 17 and Diluent 12 into aliquots appropriate to the size of your assay needs. These diluents 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 Metabolic Assay Working Solution

In a 15 mL tube combine (per plate):

- 40 µL of Aprotinin
- 240 µL of 10% Blocker D-B
- 80 µL of Blocker E
- 7640 µL of Diluent 17

**Important:** Aprotinin should be added prior to use. The Metabolic Assay Working Solution should be kept on ice. Do not freeze the Metabolic Assay Working Solution for later use.

### Prepare Calibrator and Control Solutions

Calibrator for the Human Glucagon Assay is supplied at 1 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	Glucagon conc. (pg/mL)	Dilution Factor
Stock Cal. Vial	1000000	
STD-01	10000	100
STD-02	3333	3
STD-03	1111	3
STD-04	370	3
STD-05	123	3
STD-06	41	3
STD-07	14	3
STD-08	0	n/a

To prepare this 8-point standard curve:

- 1) Prepare the highest Calibrator by transferring 10  $\mu\text{L}$  of the Calibrator stock solution at 1  $\mu\text{g}/\text{mL}$  to 990  $\mu\text{L}$  of Metabolic Assay Working Solution.
- 2) Prepare the next Calibrator by transferring 100  $\mu\text{L}$  of the diluted Calibrator to 200  $\mu\text{L}$  of Metabolic Assay Working Solution. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) The recommended 8<sup>th</sup> Standard is Metabolic Assay Working Solution (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

- 1) The assay format requires 40  $\mu\text{L}$  of sample per well. An adequate volume of each sample should be prepared depending upon desired number of replicates.
- 2) There are numerous proteases in serum and plasma that may cause degradation of Glucagon. Blood samples should be drawn into tubes containing 500 KIU Aprotinin per mL of whole blood. Alternately, Aprotinin should be added immediately following blood draw. Invert the blood tube several times to mix the sample.
  - a. To obtain serum, tubes containing Aprotinin should be allowed to clot for 30' on a rocker. Spin the tubes for 10 minutes at 1000 x g (4°C) and aliquot serum into separate tubes and store at -80°C until use. Avoid repeated freeze-thaw (> 2) of these aliquots.
  - b. Plasma samples should be obtained in vacutainer or syringe containing  $\text{Na}_2\text{EDTA}$  (1.25 mg/mL) and 500 KIU Aprotinin per mL of whole blood. Tubes should be spun for 10 minutes at 1000 x g (4°C) and then plasma immediately aliquotted into separate tubes and stored at -80°C until use. Avoid repeated freeze-thaw (> 2) of these aliquots.
- 3) Keep isolated or thawed serum/plasma samples on ice or at 4°C prior to subsequent processing or until use in the assay.
- 4) Samples with hemolysis or significant lipemia may hinder accurate assay measurements.

## 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 30  $\mu\text{L}$  aliquot of the stock Detection Antibody and 90  $\mu\text{L}$  of 10% Blocker D-B into a final volume of 3 mL of Diluent 12.

## 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  $\mu\text{L}$  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 Sample or Calibrator:** Wash the plate 3 times with PBS-T. Dispense 20  $\mu\text{L}$  of Metabolic Assay Working Solution into each well of the MSD plate. Immediately add 40  $\mu\text{L}$  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 Addition of the Detection Antibody Solution:** Wash the plate 3 times with PBS-T. Dispense 25  $\mu\text{L}$  of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
4. **Wash and Read:** Wash the plate 3 times with PBS-T. Add 150  $\mu\text{L}$  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-SPOT plate typically accelerates capture at the working electrode.*

*Bubbles in the fluid will interfere with reliable reading of MULTI-SPOT 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<sup>®</sup> 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.

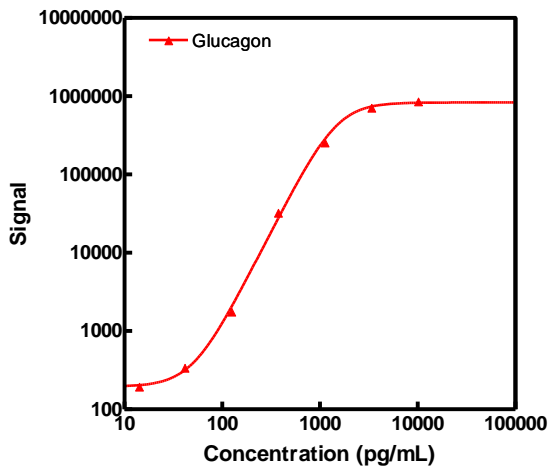
## IX

# Typical Standard Curve

typical standard curve

The MSD Human Glucagon Assay is designed for use with human 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.



Glucagon		
Conc. (pg/mL)	Average Signal	%CV
0	152	12.1
14	196	7.9
41	342	5.0
123	1781	3.1
370	32710	7.0
1111	258830	7.2
3333	717347	4.2
10000	867267	5.2

## 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.

Glucagon	
LLOD (pg/mL)	27

# 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 4-6 pooled human serum and plasma samples. MSD recommends using plasma samples for optimal assay performance.

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

	Spike Conc. (pg/mL)	% Recovery
<b>Spiked Serum</b>	100	64
	500	70
	1000	70
<b>Spiked EDTA Plasma</b>	100	88
	500	79
	1000	83
<b>Spiked Heparin Plasma</b>	100	85
	500	80
	1000	82

# XII Linearity

linearity

Linearity was measured by spiking Calibrator levels in pooled human 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
<b>EDTA Plasma</b>	2	102
	4	119
	8	127
<b>Heparin Plasma</b>	2	107
	4	104
	8	104

# XIII Assay Components

## assay components

Calibrator	
<b>Analyte</b>	Glucagon
<b>Source</b>	Synthetic human glucagon (amino acids 1-29)

Capture Antibody	
<b>Analyte</b>	Human glucagon
<b>Source</b>	Mouse monoclonal
<b>Isoforms Recognized</b>	n/a
<b>Species cross-reactivity</b>	Human, mouse, rat

Detection Antibody	
<b>Analyte</b>	Human glucagon
<b>Source</b>	Mouse monoclonal, ascites
<b>Isoforms Recognized</b>	Pancreatic glucagon, reacts weakly to gut glucagon
<b>Species cross-reactivity</b>	Human, mouse, rat, sheep, rabbit, pig, canine, pig, guinea pig

# XIV References

## references

1. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF. Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem.* 1986 Sep 5;261(25):11880-9.
2. Witt S, Dietz H, Ziegler B, Keilacker H, Ziegler M. Production and use of monoclonal glucagon and insulin antibodies - reduction of pancreatic insulin in rats by treatment with complete Freund's adjuvant. *Acta Histochem Suppl.* 1988;35:217-23
3. Ahrén B. Glucagon secretion in relation to insulin sensitivity in healthy subjects. *Diabetologia.* 2006 Jan;49(1):117-22



## Summary Protocol

### MSD 96-well MULTI-ARRAY Human Glucagon Assay Kit

MSD provides this summary protocol for your convenience.  
Please read the entire detailed protocol prior to performing the Human Glucagon 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 Metabolic Assay Working Solution and keep on ice.

Prepare an 8-point standard curve using supplied Calibrator:

- The Calibrator should be diluted in Metabolic Assay Working Solution.
- Dilute the stock Calibrator 1:100 as indicated in Reagent Preparation section, 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-Glucagon Antibody to 1X and the 10% Blocker D-B to 0.3% in 3.0 mL of Diluent 12 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  $\mu$ L/well Blocker A Solution.

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

#### Step 3 : Wash and Add Sample or Calibrator

Wash plate 3 times with PBS-T.

Dispense 20  $\mu$ L/well Metabolic Assay Working Solution.

Immediately, dispense 40  $\mu$ L/well Calibrator or Sample.

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

#### Step 4 : Wash and Add Detection Antibody Solution

Wash plate 3 times with PBS-T.

Dispense 25  $\mu$ L/well 1X Detection Antibody Solution.

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

#### Step 5 : Wash and Read Plate

Wash plate 3 times with PBS-T.

Dispense 150  $\mu$ L/well 1X Read Buffer T.

Analyze plate on SECTOR instrument.



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