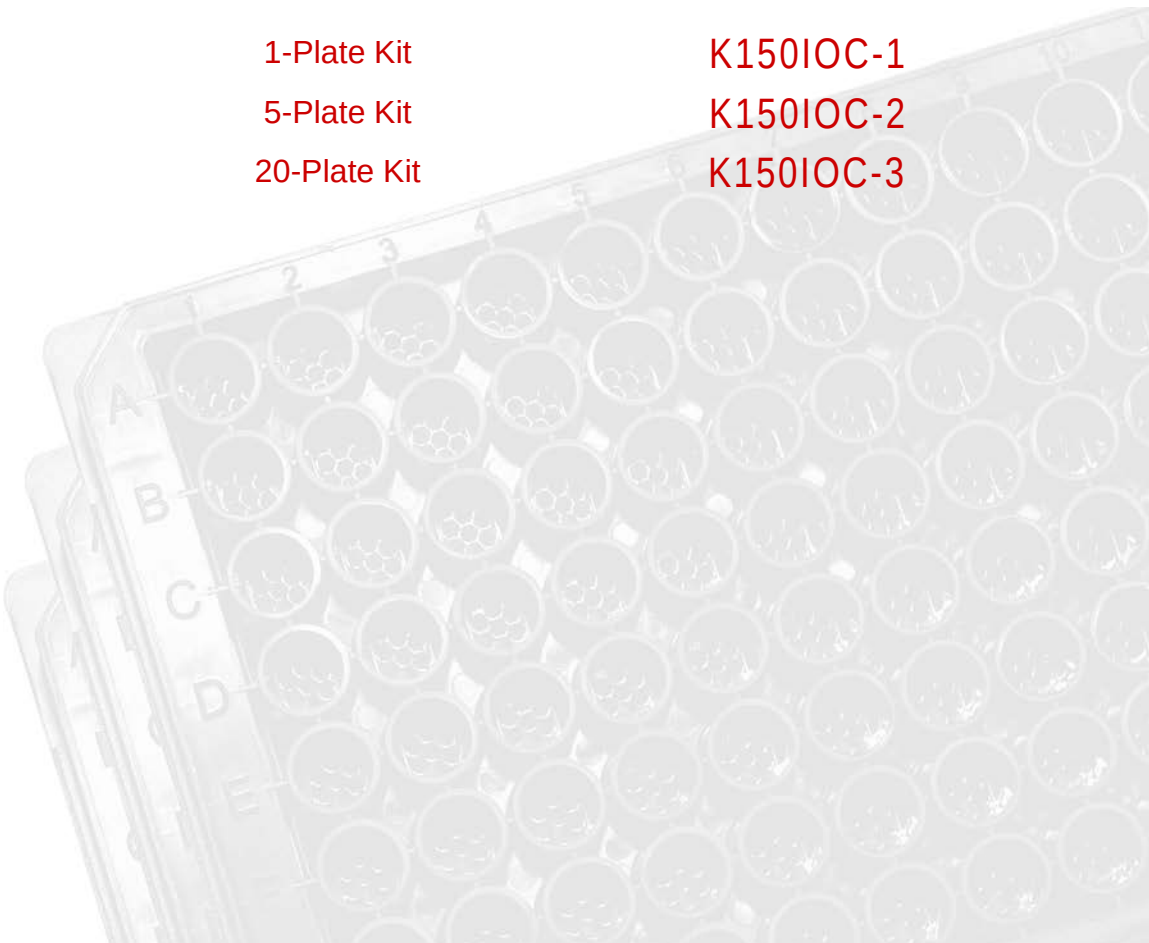
Meso Scale Discovery®

MULTI-ARRAY® Assay System

Mouse/Rat Total Ghrelin Kit

1-Plate Kit	K150IOC-1
5-Plate Kit	K150IOC-2
20-Plate Kit	K150IOC-3



MSD Metabolic Assays

Mouse/Rat Total Ghrelin 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.
9238 Gaither Road
Gaithersburg, MD 20877 USA
www.mesoscale.com

Table of Contents

table of contents

Ordering Information

ordering information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

introduction

Ghrelin is a 28 residue gut hormone produced mainly by P/D1 cells lining the fundus of the stomach and epsilon cells of the pancreas that stimulates appetite. Bioactivity of this peptide hormone is dependent on n-octanoylation at the third reside (Serine) and this active form is the endogenous ligand of the GH secretagoge receptor (GHS-R). Octanoylation is considered essential for activity, and the des-octanolylated ghrelin is inactive. Ghrelin acts as the counterpart to the hormone leptin, produced by adipose tissue, which induces satiation when present at higher levels. In contrast, ghrelin levels increase before meals and decrease after meals. Administration of ghrelin to animals stimulates gastric acid secretion and motility, increasing food intake, weight gain and reduced fat utilization. Ghrelin levels are low in obese and high in lean individuals, suggesting that ghrelin is important not only for appetite but also in long-term regulation of energy homeostasis.

Ghrelin is also produced in the hypothalamic arcuate nucleus where it stimulates the secretion of growth hormone from the anterior pituitary gland. In some bariatric procedures, the level of ghrelin is reduced in patients, thus causing satiation before it would normally occur. Ghrelin also plays a significant role in neurotrophy, particularly in the hippocampus, and is essential for cognitive adaptation to changing environments and the process of learning.

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 Total Ghrelin Assay detects acylated and desacylated forms of Ghrelin in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with Ghrelin capture antibody. The user adds the sample and a solution containing the labeled detection antibody—anti-Ghrelin labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. Ghrelin 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 acylated and desacylated forms of Ghrelin present in the sample.

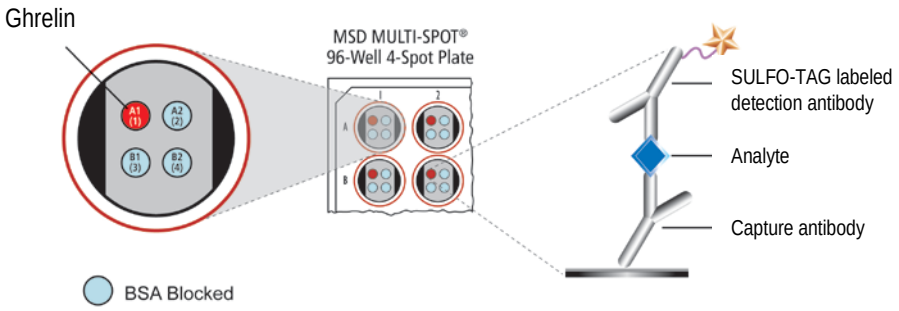


Figure 1. *Sandwich immunoassay on MSD platform. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding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

III Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit K150IOC-1	K150IOC-2	K150IOC-3
MULTI-SPOT 96-well Mouse/Rat Ghrelin Plate(s) N450FAA-1	2-8°C	1 plate	5 plates	20 plates
SULFO-TAG Anti-m/r Ghrelin Antibody[1] (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	4 vials (200 µL ea)
Rat Des-Ghrelin Calibrator 50 µg/mL	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	20 vials (20 µL ea)
Blocker A Kit R93AA-2 (250 mL)	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)
Diluent 13 R56BB-3 (50 mL)	≤-10°C	1 bottle (50 mL)	1 bottle (50 mL)	4 bottles (50 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)

IV 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

V 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 13 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 Assay Diluent Working Solution

In a 15 mL tube combine (per plate):

- ❑ 40 µL of Aprotinin
- ❑ 7960 µL of Diluent 13

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

Prepare Calibrator and Control Solutions

Calibrator for the Total Ghrelin Assay is supplied at 50 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	Des-Ghrelin conc. (pg/mL)	Dilution Factor
Stock Cal. Vial	50000000	
Diluted Cal. Vial	500000	100
STD-01	5000	100
STD-02	1667	3
STD-03	555	3
STD-04	185	3
STD-05	62	3
STD-06	21	3
STD-07	6.8	3
STD-08	0	n/a

To prepare this 8-point standard curve:

1) Dilute the Rat Des-Ghrelin Calibrator to a concentration of 0.5 µg/mL. Add 5 µL of the Calibrator stock solution at 50 µg/mL to 495 µL of Assay Diluent Working Solution.
2) Prepare the highest Calibrator by adding 5 µL of the diluted Calibrator solution at 0.5 µg/mL to 495 µL of Assay Diluent Working Solution.
3) Prepare the next Calibrator by transferring 100 µL of the highest Calibrator to 200 µL of Assay Diluent Working Solution. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
4) The recommended 8th Standard is Assay Diluent Working Solution (i.e. zero Calibrator).
5) 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 25 µL 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 Ghrelin. 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 Na_2EDTA (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 µL aliquot of the stock Detection Antibody into 2.97 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 µL 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 25 µL of Assay Diluent Working Solution into each well of the MSD plate. Immediately add 25 µL 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 µL 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 µL 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

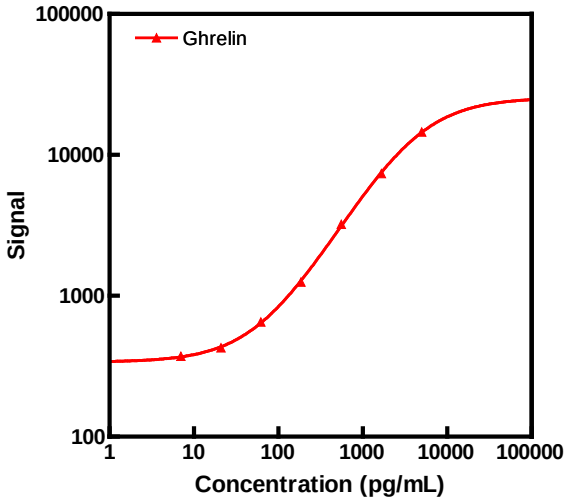
The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantification in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH® 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 Total Ghrelin Assay is designed for use with mouse or 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.



	Total Ghrelin	
Conc. (pg/mL)	**Average Signal**	**%CV**
0	319	5
7	372	13
21	427	2
62	652	1
185	1250	1
556	3218	1
1667	7362	3
5000	14525	8

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.

	Total Ghrelin
LLOD (pg/mL)	11

XI Endogenous Levels

Endogenous levels

Endogenous levels of Total Ghrelin measured from 5-8 individual mouse and rat serum and plasma samples.

	Rat			Mouse	
Sample	**Serum (pg/mL)**	**EDTA Plasma (pg/mL)**	**Heparin Plasma (pg/mL)**	**Serum (pg/mL)**	**ob/ob Serum (pg/mL)**
Average	111	255	242	70	13
Range	85-160	170-348	178-294	23-183	0-32

XII 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 3 pooled mouse serum and plasma samples.
% Recovery = measured /expected x 100

	Spike Conc. (µg/mL)	**% Recovery**
Spiked Serum	1000	95
	5000	92
	10000	98
Spiked EDTA Plasma	1000	99
	5000	105
	10000	96
Spiked Heparin Plasma	1000	94
	5000	91
	10000	96

XIII Linearity

linearity

Linearity was measured by spiking Calibrator levels in pooled mouse serum and plasma followed by subsequent dilution.
Values presented are averages of 3 samples.
Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).
% Recovery = measured x dilution factor / expected x 10

	Fold Dilution	% Recovery
Serum	2	104
	4	109
	8	112
EDTA Plasma	2	102
	4	104
	8	88
Heparin Plasma	2	113
	4	103
	8	112

XIV Assay Components

assay components

Calibrator	
Analyte	Rat [Ser3(Des-Octanoyl)] ghrelin
Source	Synthetic peptide (amino acids 1-28); 100% homology to mouse sequence

Capture Antibody	
Analyte	Ghrelin
Source	Chicken polyclonal
Isoforms Recognized	Ser3(O-n-Octanolylated) and Ser3(Des-Octanoyl) Ghrelin
Species cross-reactivity	Human, mouse, rat

Detection Antibody	
Analyte	Ghrelin
Source	Chicken polyclonal
Isoforms Recognized	Ser3(O-n-Octanolylated) and Ser3(Des-Octanoyl) Ghrelin
Species cross-reactivity	Human, mouse, rat

XV References

references

1. Wortley KE, Anderson KD, Garcia K, et al. Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. Proc Natl Acad Sci U S A. 2004 May 25;101(21):8227-32

2. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K.Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999 Dec 9;402(6762):656-60

3. Davenport AP, Bonner TI, Foord SM, Harmar AJ, Neubig RR, Pin JP, Spedding M, Kojima M, Kangawa K.International Union of Pharmacology. LVI. Ghrelin receptor nomenclature, distribution, and function. Pharmacol Rev. 2005 Dec;57(4):541-6

4. Gelling RW, Overduin J, Morrison CD, Morton GJ, Frayo RS, Cummings DE, Schwartz MW. Effect of uncontrolled diabetes on plasma ghrelin concentrations and ghrelin-induced feeding. Endocrinology. 2004 Oct;145(10):4575-82

5. De Vriese C, Gregoire F, Lema-Kisoka R, Waelbroeck M, Robberecht P, Delporte C. Ghrelin degradation by serum and tissue homogenates: identification of the cleavage sites. Endocrinology. 2004 Nov;145(11):4997-5005

Summary Protocol

MSD 96-well MULTI-ARRAY Mouse/Rat Total Ghrelin Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Mouse/Rat Total Ghrelin 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 Assay Diluent Working Solution and keep on ice.

Prepare an 8-point standard curve using supplied Calibrator:

- The Calibrator should be diluted in Assay Diluent Working Solution.
- Dilute the stock Calibrator 1:10000 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-m/r Ghrelin Antibody to 1X 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 µL/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 25 µL/well Assay Diluent Working Solution.

Immediately, dispense 25 µL/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 µL/well 1X Detection Antibody Solution.

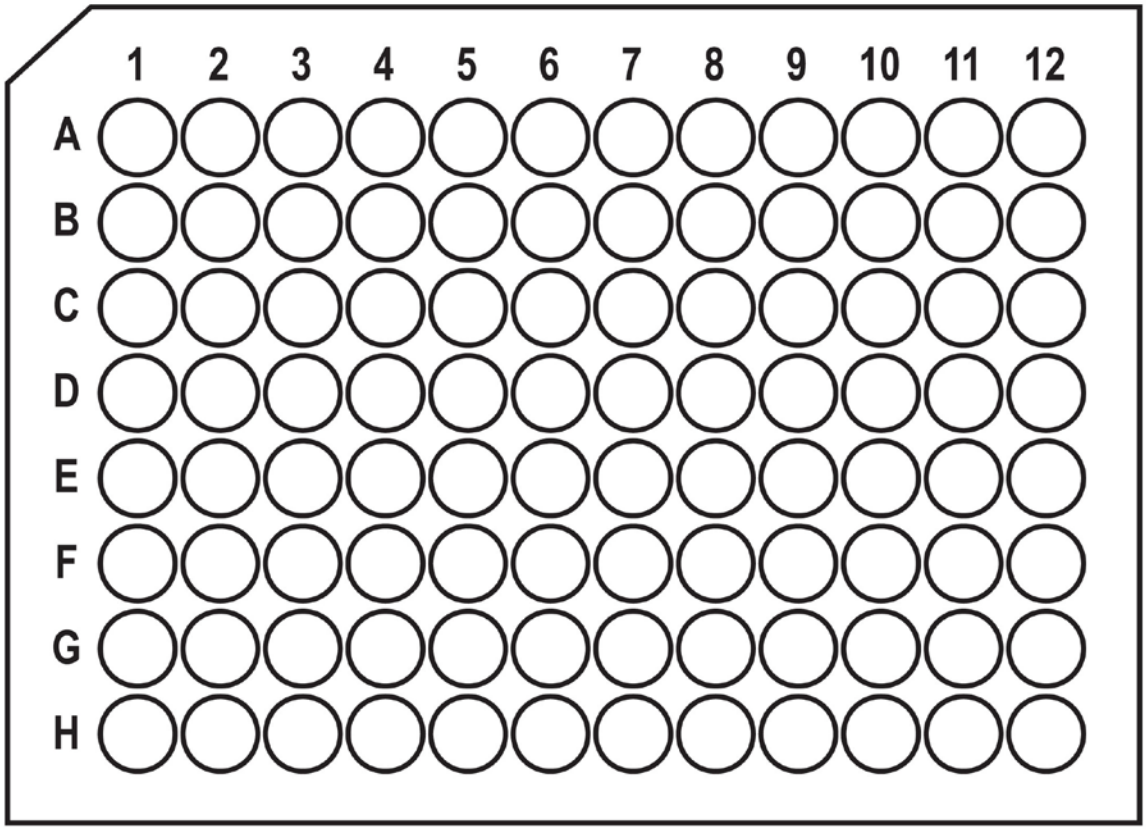
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

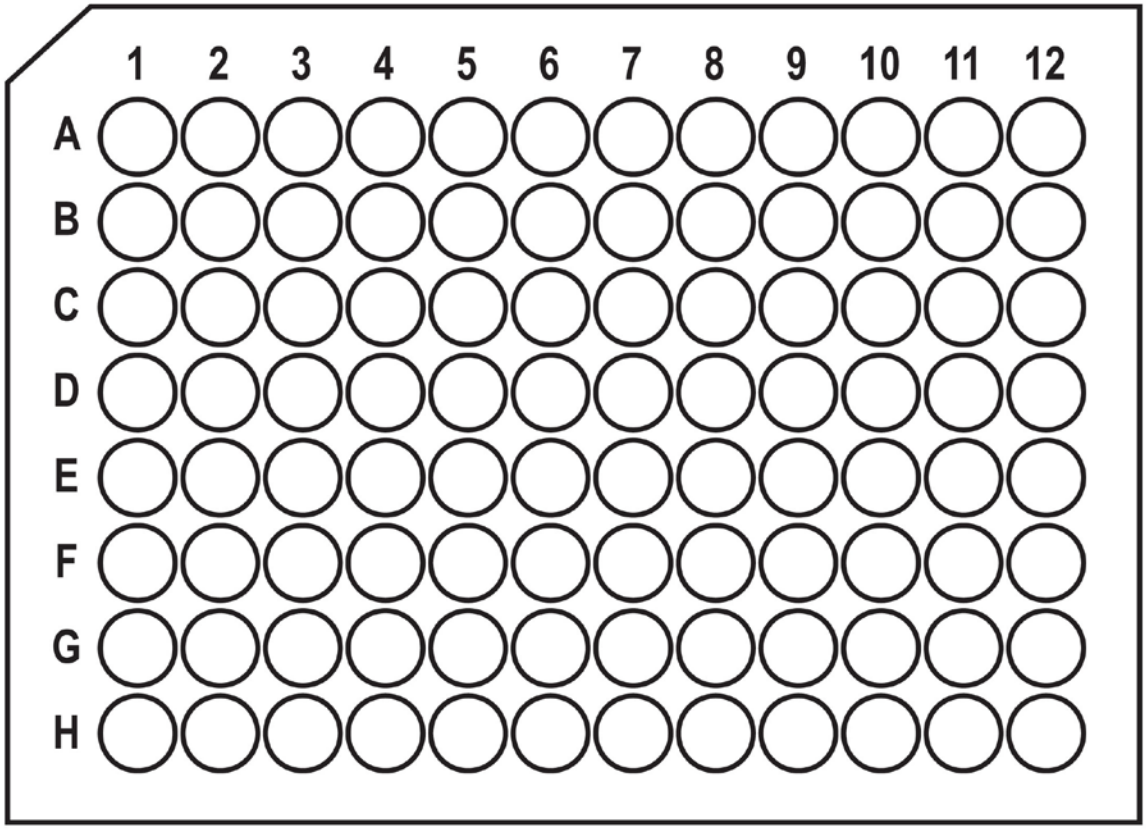
Step 5 : Wash and Read Plate

Wash plate 3 times with PBS-T.

Dispense 150 µL/well 1X Read Buffer T.

Analyze plate on SECTOR instrument.


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H


1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
F
G
H