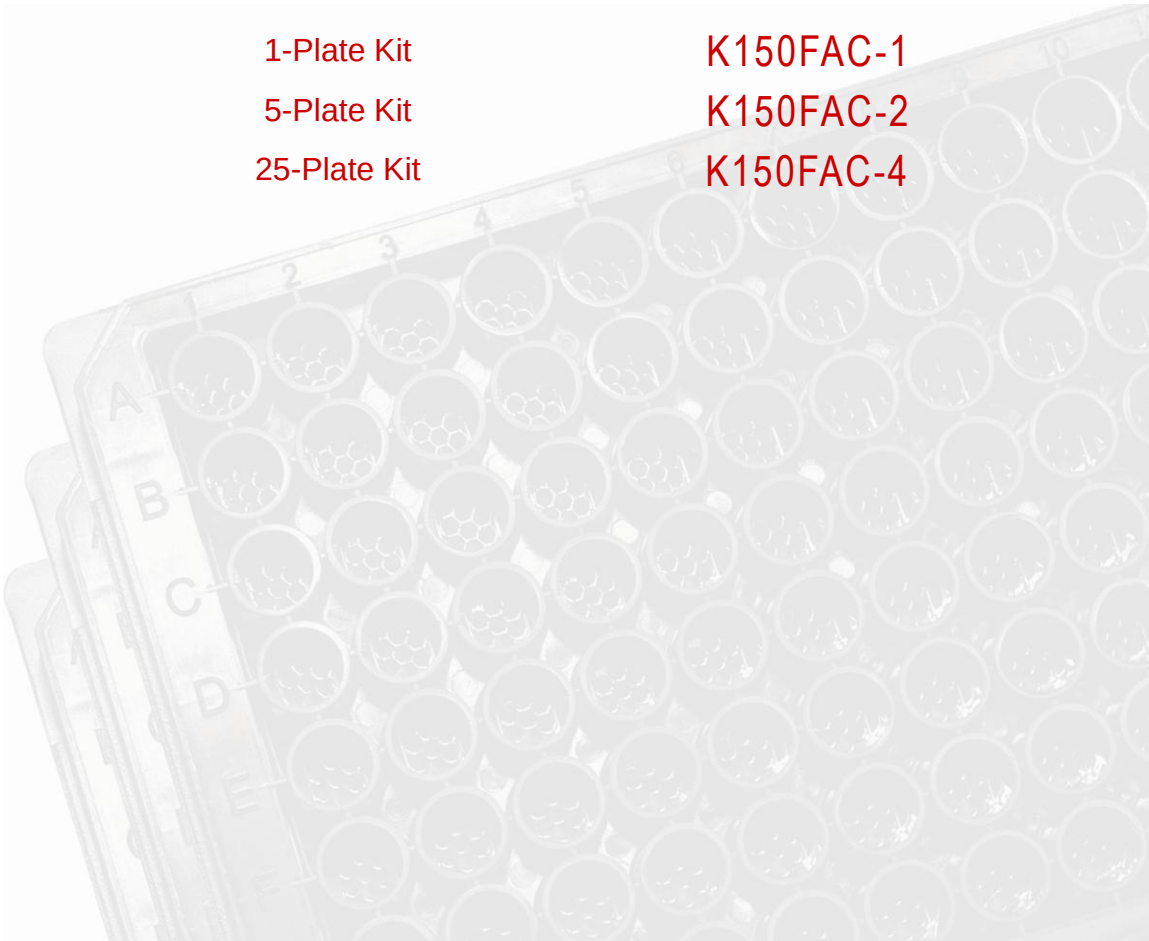
MESO SCALE DISCOVERY

MULTI-ARRAY® Assay System

GLP-1 (x-36) amide Assay Kit

1-Plate Kit	K150FAC-1
5-Plate Kit	K150FAC-2
25-Plate Kit	K150FAC-4



MSD Metabolic Assays

GLP-1 (x-36) amide 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.

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Ordering Information

ordering information

MSD Customer Service

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Introduction

introduction

Glucagon-like peptide-1 (GLP-1), a post-translational product of preproglucagon, is a 3.5 kD protein hormone produced in intestinal L cells and plays a key role in the promotion of glucose-dependent insulin secretion and insulin biosynthesis. In addition, GLP-1 works in concert with insulin to inhibit glucose secretion and thus lower overall blood glucose levels. Through the activation of different physiological systems, it plays roles in gastric emptying upon nutrient intake and in the regulation of short-term feeding behavior. Upon release, its action is mediated through a single G-protein-coupled receptor. GLP-1 receptors have been identified in several tissues, including pancreatic islets, lung, gastrointestinal tract and the central nervous system (CNS). Emerging evidence suggests GLP-1 also can provide beneficial, cyto-protective effects on neuronal cells, skeletal muscle and the myocardium, elevating its potential role in Alzheimer's and cardiovascular homeostasis and disease.

The cleaved peptides, commonly referred to as GLP-1 (7-36) amide and GLP-1 (7-37) are the biologically active forms of GLP-1. *In vivo*, these active isoforms are rapidly cleaved by dipeptidyl peptidase IV (DPP IV). Since GLP-1, in its bioactive form, plays a crucial role in blood glucose regulation, GLP-1 mimetics and inhibitors of DPP IV are currently being evaluated as potential drug candidates in treatment of diabetes. The primary amino acid sequence for GLP-1 is conserved among mammalian species, i.e. human, mouse, rat, monkey, canine, etc.

MSD offers a comprehensive array of GLP-1 assays that measure active, total and amidated isoforms of the GLP-1 protein using detection antibodies specific for the C-terminal amino acids.

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 GLP-1 (x-36) amide Assay detects all amidated forms of GLP-1 in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with GLP-1 total capture antibody. The user adds the sample and a solution containing the labeled detection antibody—anti-GLP-1 (7-36) amide labeled with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. GLP-1 (x-36) amide 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 all amidated forms of GLP-1 {isoforms:(1-36), (7-36) and (9-36)} present in the sample (Figure 2).

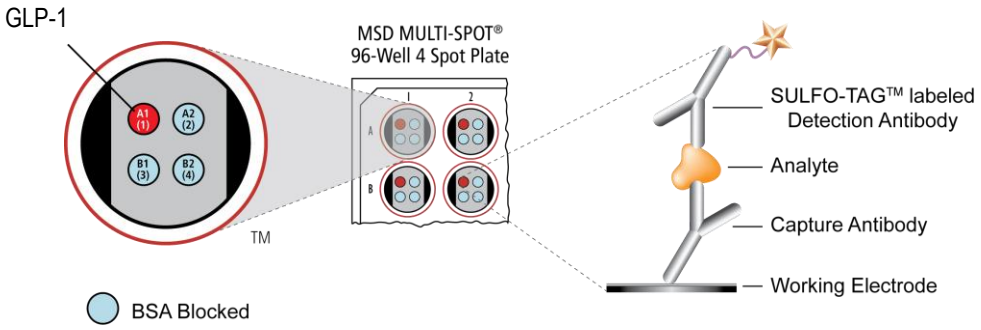


Figure 1. *Sandwich immunoassay on MSD platform. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding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

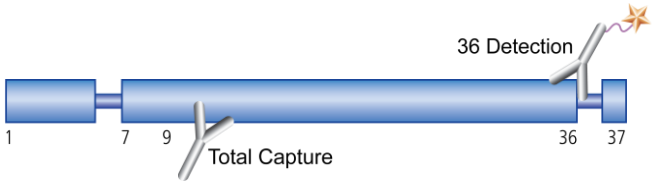


Figure 2. *Schematic of the antibody recognition sites for GLP-1 (x-36) amide Assay on GLP-1 protein amino acids 1-37.*

III Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit K150FAC-1	K150FAC-2	K150FAC-4
MULTI-SPOT 96-well GLP-1 Plate(s) N450FAA-1	2-8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-GLP-1 (7-36) amide Antibody[1] (100X)	2-8°C	1 vial (40 µL)	1 vial (200 µL)	5 vials (200 µL ea)
GLP-1 (7-36) amide Calibrator 1 µg/mL	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (250 mL ea)
Aprotinin (200,000 KIU/mL)	2-8°C	1 vial (50 µL)	1 vial (250 µL)	5 vials (250 µL ea)
Diluent 13 R56BB-4 (10 mL) R56BB-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 12 R50JA-4 (10 mL) R50JA-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

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 Metabolic Assay 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 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 GLP-1 (x-36) amide Assay is supplied at 1 µg/mL. For the assay, an 8-point standard curve is recommended with 4-fold serial dilution steps and a zero Calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	GLP-1 (7-36) amide conc. (pg/mL)	Dilution Factor
Stock Cal. Vial	1000000	
STD-01	10000	100
STD-02	2500	4
STD-03	625	4
STD-04	156	4
STD-05	39	4
STD-06	9.8	4
STD-07	2.4	4
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 solution at 1 µg/mL to 990 µL of Metabolic Assay Working Solution.
2) Prepare the next Calibrator by transferring 75 µL of the diluted Calibrator to 225 µL of Metabolic Assay Working Solution. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
3) The recommended 8th 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

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.

a) Samples can be collected using BD™ P800 Blood Collection and Preservation System which contains DPP-IV and other protease inhibitor cocktails (Product Number 366420). Alternatively, whole blood should be collected into a lavender top BD Vacutainer® EDTA-plasma tube (Product Number 367841). DPP-IV inhibitor and Aprotinin should be added immediately to avoid cleavage/degradation of GLP-1. Spin the tubes for 10 minutes at 1000 x g (4°C).
b) Samples can be stored at 2-8ºC if used within 3 hours. Samples for later use should immediately be aliquotted into separate tubes and stored at ≤-70°C. Avoid repeated freeze-thaw (> 2) of these aliquots.
c) Keep isolated or thawed samples on ice or at 4°C prior to subsequent processing or until use in the assay.
d) 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 Metabolic Assay Working Solution into each well of the MSD plate. Immediately 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

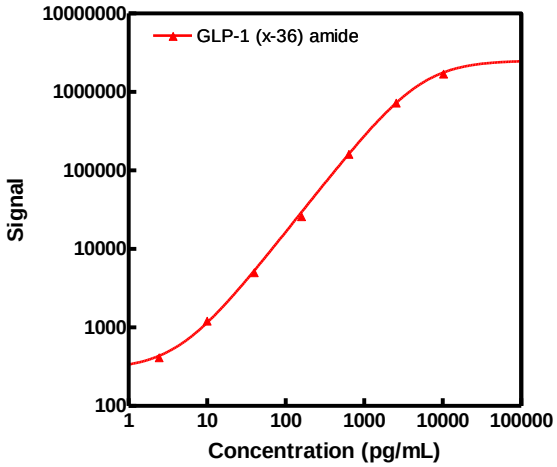
The Calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have 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 GLP-1 (x-36) amide Assay is designed for use with human, 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.



	GLP-1 (x-36) amide	
Conc. (pg/mL)	**Average Signal**	**%CV**
0	153	4.2
2.4	420	3.6
9.8	1234	5.9
39	5081	2.6
156	26339	2.3
625	164224	3.3
2500	740128	3.3
10000	1724514	3.8

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.

1 pmol/L = 3.297pg/mL

	GLP-1 (x-36) amide
LLOD (pg/mL)	0.47

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 mouse or human samples. MSD recommends using plasma samples for optimal assay performance.
% Recovery = measured /expected x 100

		% Recovery	
	Spike Conc. (pg/mL)	Human	Mouse
Spiked Serum	100	65	96
	500	78	105
	1000	86	99
Spiked EDTA Plasma	100	70	112
	500	82	123
	1000	85	136
Spiked Heparin Plasma	100	79	93
	500	95	96
	1000	93	104

XII Linearity

linearity

Linearity was measured by spiking Calibrator levels in pooled mouse serum or human EDTA plasma followed by subsequent dilution.
Percent recovery is calculated as the measured concentration divided by the concentration of the previous dilution (expected).
% Recovery = measured x dilution factor / expected x 100

		% Recovery	
	Fold Dilution	Mouse	Human
Serum	2	106	-
	4	111	-
	8	113	-
Spiked Serum	2	106	-
	4	114	-
	8	123	-
Spiked EDTA Plasma	2	-	97
	4	-	93
	8	-	95

XIII Cross-Reactivity

cross-reactivity

The cross-reactivity shown below is calculated based on signal generated using different GLP-1 isoforms.

GLP-1 (x-36) amide	
Form	**Cross-Reactivity**
GLP-1 (7-36) amide	100%
GLP-1 (9-36) amide	52%
GLP-1 (1-36) amide	97%
GLP-1 (7-37)	<1%
GLP-1 (1-37)	<1%

XIV Assay Components

assay components

Calibrator	
Analyte	GLP-1 (7-36) amide
Source	Synthetic amidated peptide (amino acids 7-36) of human GLP-1

Capture Antibody	
Analyte	GLP-1
Source	Mouse monoclonal
Isoforms Recognized	Reacts with all forms of GLP-1, including precursor
Species cross-reactivity	Human, mouse, rat (100% conserved in all mammalian species)

Detection Antibody	
Analyte	GLP-1
Source	Mouse monoclonal
Isoforms Recognized	Reacts with the amidated C terminus of GLP-1 (7-36) amide, GLP-1 (9-36) amide and GLP-1 (1-36) amide, does not react with GLP-1 (7-37), GLP-1 (9-37) or GLP-1 (1-37)
Species cross-reactivity	Human, mouse, rat (100% conserved in all mammalian species)

XV References

references

1. Kieffer TJ, Habener JF. The Glucagon-Like Peptides. Endo Reviews. 2006 Dec;20(6):876-913

2. Pospisilik JA, Martin J, Doty T, et al. Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. Diabetes. 2003 Mar;52(3):741-50.

3. Baggio LL, Drucker DJ**.** Biology of incretins: GLP-1 and GIP. Gastroenterology. 2007 May;132(6):2131-57.

4. Holst JJ, Deacon CF. Glucagon-like peptide 1 and inhibitors of dipeptidyl peptidase IV in the treatment of type 2 diabetes mellitus. Curr Opin Pharmacol. 2004 Dec;4(6):589-96

5. Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. J Clin Endocrinol Metab. 1995 Mar;80(3):952-7

Summary Protocol

MSD 96-well MULTI-ARRAY GLP-1 (x-36) amide Assay Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the GLP-1 (x-36) amide 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 4-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-GLP-1 (7-36) amide 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 Metabolic Assay 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.

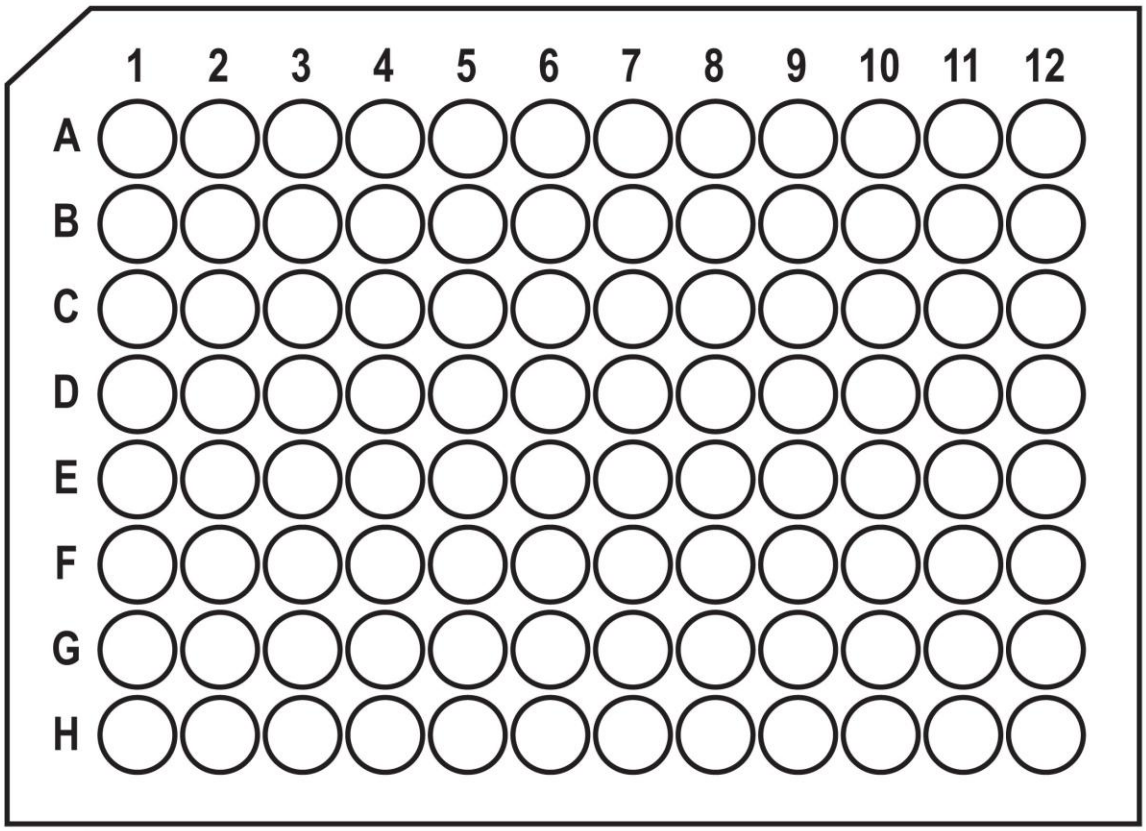
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

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.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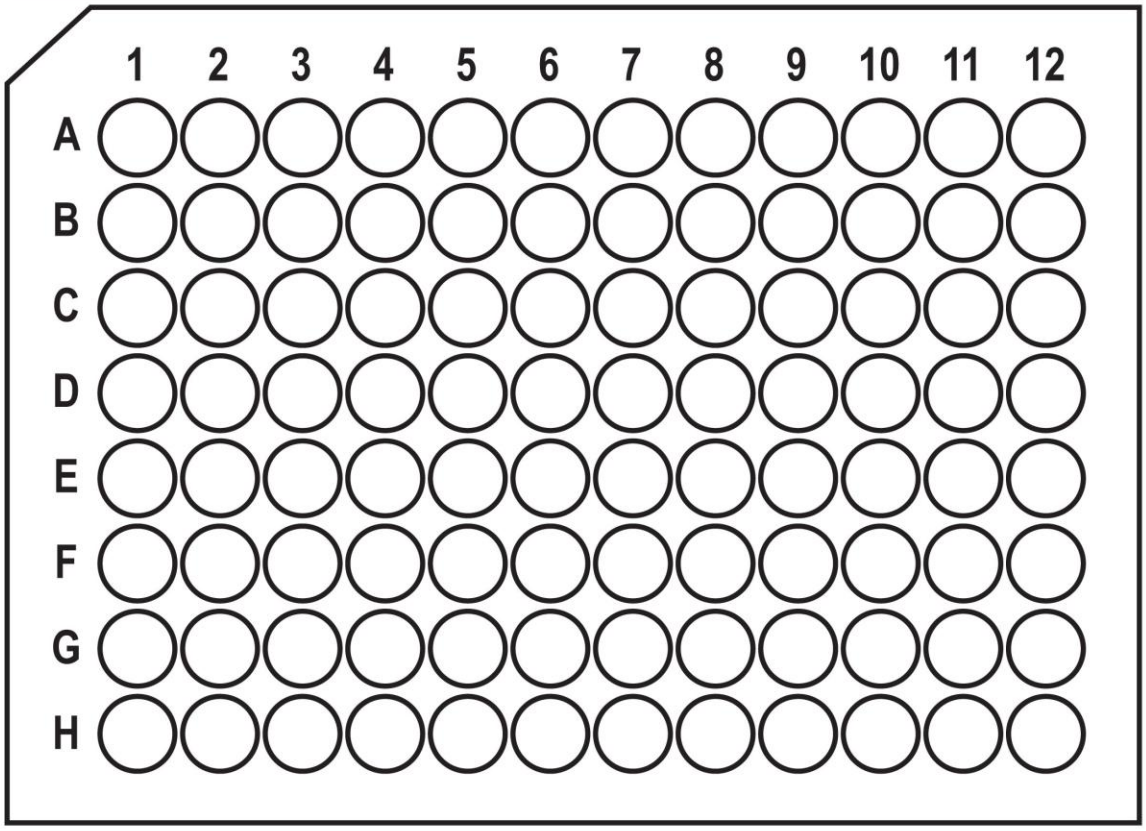
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