MSD® MULTI-ARRAY Assay System

Human Total PYY Kit

1-Plate Kit	K151MPD-1
5-Plate Kit	K151MPD-2
25-Plate Kit	K151MPD-4

www.mesoscale.com®

MSD Metabolic Assays

Human Total PYY 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.
1601 Research Blvd.
Rockville, MD 20850 USA
www.mesoscale.com

Table of Contents

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

Peptide-YY (PYY) is a 36-amino acid peptide secreted from the intestinal L-cells of the gastrointestinal tract in proportion to the caloric content of a meal. Plasma PYY levels rise within 15 minutes after starting to eat and plateau within approximately 90 minutes, remaining elevated for up to 6 hours.[1] Two endogenous forms, PYY(1-36) and PYY(3-36), are released into circulation; PYY(3-36) is the major form (60%) of PYY in gut mucosal endocrine cells and in the blood stream. This form results from the cleavage of PYY by the enzyme dipeptidyl peptidase-IV (DPP-IV); it acts on neuropeptide Y (NPY) receptors in the CNS to mediate satiety within the hypothalamic arcuate nucleus.[2,3] Exogenous administration of PYY(3-36) reduces energy intake and body weight in both humans and animals. Some studies have reported that obese individuals have lower basal fasting levels of PYY(3-36) and have a smaller rise in postprandial levels suggesting PYY may be a potential therapeutic target in obesity.[1-3]

Principle of the Assay

MSD metabolic assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human Total PYY is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with streptavidin, and the user adds human PYY capture antibody conjugated to biotin. The user then adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Biotin-conjugated capture antibody binds to the streptavidin that has been immobilized on the working electrode surface, analytes in the sample bind to capture antibodies, and the recruitment of the labeled detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into a SECTOR® Imager where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample. The assay measures both the 1-36 and 3-36 forms of PYY.

Analyte: PYY

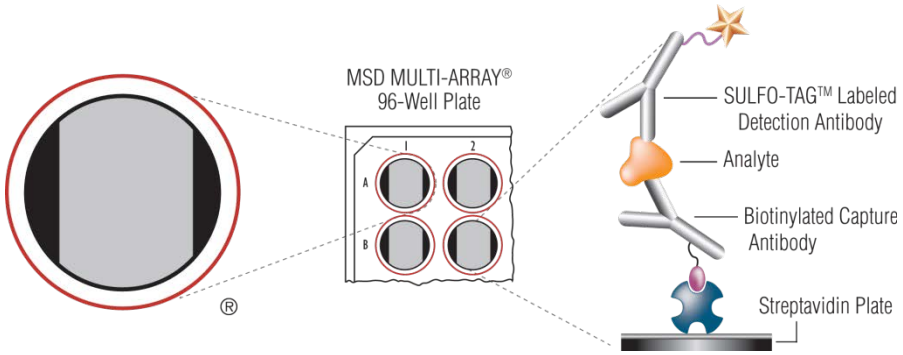


Figure 1. Spot diagram showing placement of analyte capture antibodies. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K151MPD-1	K151MPD-2	K151MPD-4
MULTI-ARRAY 96-Well STREPTAVIDIN GOLD™ Plate L15SA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu PYY Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Anti-hu PYY Biotinylated Capture Antibody (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human PYY Calibrator (0.06 µg/mL)	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 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 100 R50AA-4 (50 mL), R50AA-2 (200 mL)	2–8°C	1 bottle (50 mL)	1 bottle (200 mL)	2 bottles (200 mL ea)
Aprotinin (200 000 KIU/mL)	2–8°C	1 vial (250 µL)	2 vials (250 µL ea)	4 vials (250 µL ea)
Blocker D-B[2] (10%)	≤-10°C	1 vial (1.2 mL)	2 vials (1.2 mL ea)	4 vials (1.2 mL ea)
Blocker A Kit (Blocker A [dry] in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer) R93AA-2 (250 mL)	RT	1 kit (250 mL)	1 kit (250 mL)	5 kits (250 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Required Material and Equipment (not supplied)

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Deionized water

[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.
[2] Blocker D-B can tolerate up to 5 freeze-thaw cycles. Alternatively, an aliquot of the blocker can be stored at 2–8°C up to 1 month.

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 3 into aliquots appropriate for the size of your needs before refreezing.

Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Metabolic Assay Diluent Working Solution

MSD provides aprotinin stock solution at a concentration of 200 000 KIU/mL. The working solution is 1000 KIU/mL.

For 1 plate, combine:

- ❑ 50 µL of Aprotinin
- ❑ 9.95 mL of Diluent 13

Important: Aprotinin should be added prior to use. The metabolic assay diluent working solution should be kept on ice. Do not freeze solution for later use.

Prepare Capture Antibody Solution

MSD provides biotinylated capture antibody as a 50X stock solution. The working capture antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X Anti-hu PYY Biotinylated Capture Antibody
- ❑ 2.94 mL of metabolic assay diluent working solution

Prepare Standards

MSD supplies calibrator for the Human Total PYY Kit at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 2-fold serial dilution steps and a zero calibrator blank. Signals from the blank should be excluded when generating the curve. Thaw the stock calibrator and keep on ice. Prepare the standard solutions at room temperature.

Standard	PYY Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	60 000	
STD-01	3000	20
STD-02	1500	2
STD-03	750	2
STD-04	375	2
STD-05	188	2
STD-06	94	2
STD-07	47	2
STD-08	0	n/a

To prepare 7 standard solutions plus a zero calibrator blank for up to 4 replicates:

1) Prepare the highest standard (STD-01) by adding 20 µL of stock calibrator to 380 µL of metabolic assay diluent working solution. Mix well.
2) Prepare the next standard (STD-02) by transferring 200 µL of STD-01 to 200 µL of metabolic assay diluent working solution. Mix well. Repeat 2-fold serial dilutions 5 additional times to generate 7 standards.
3) Use metabolic assay diluent working solution as the blank.

Dilute Samples

Serum and plasma samples are recommended without any dilutions for this assay. Avoid multiple freeze–thaw cycles.

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution and Blocker D-B as a 10% stock solution. The working detection antibody solution is 1X with 0.3% Blocker D-B .

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu PYY Antibody
- ❑ 90 µL of Blocker D-B
- ❑ 2850 µL of Diluent 100

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X.

For 1 plate, combine:

- ❑ 5 mL of Read Buffer T (4X)
- ❑ 15 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

Each lot of MSD STREPTAVIDIN GOLD plates (Figure 1) passes rigorous inter- and intra- plate quality control specifications for consistency, uniformity, and binding capacity as reflected in the lot-specific certificate of analysis. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Notes

1. **Add Blocker A Solution:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   Shaking the plate typically accelerates capture at the working electrode.

2. **Wash and Add Capture Antibody Solution and Sample:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of 1X Capture Antibody. Add 25 µL of sample (standards, controls, or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

   You may prepare diluted read buffer during incubation.

   Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 1X Read Buffer T to each well. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

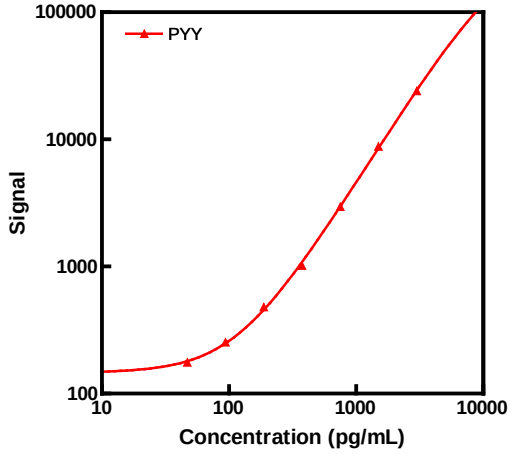
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate the standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification without the need for dilution in many cases. By default, the software uses a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Data

The following standard curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of standards.



PYY		
Conc. (pg/mL)	Average Signal	%CV
0	138	8.2
47	176	6.3
94	254	3.1
188	480	3.5
375	1016	12.7
750	2958	3.5
1500	8810	4.9
3000	24 037	4.2

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank).

	PYY
Average LLOD (pg/mL)	68

Assay Components

Calibrator

The assay calibrator uses synthesized human PYY (amino acids 3-36) peptide.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
PYY	Mouse Monoclonal	Rabbit Polyclonal

References

1. Beglinger C, Degen L. Gastrointestinal satiety signals in humans--physiologic roles for GLP-1 and PYY? Physiol Behav. 2006 Nov 30;89(4):460-4.
2. Zac-Varghese S, et al. Translational studies on PYY as a novel target in obesity. Curr Opin Pharmacol. 2011 Dec;11(6):582-5.
3. De Silva A, Bloom SR. Gut Hormones and Appetite Control: A Focus on PYY and GLP-1 as Therapeutic Targets in Obesity. Gut Liver. 2012 Jan;6(1):10-20.

Summary Protocol

Human Total PYY Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human Total PYY assay.

Sample and Reagent Preparation

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

Prepare Blocker A solution.

Prepare metabolic assay diluent working solution by diluting the stock aprotinin 200-fold in Diluent 13.

Prepare capture antibody solution by diluting the stock capture antibody 50-fold in metabolic assay diluent working solution.

Prepare 7 standard solutions using the supplied calibrator:

- Dilute the stock calibrator 20-fold in metabolic assay diluent working solution.
- Perform a series of 2-fold dilution steps and prepare a zero calibrator blank.

Prepare detection antibody solution by diluting the stock detection antibody 50-fold in Diluent 100 containing 0.3% Blocker D-B.

Prepare 1X Read Buffer T by diluting stock 4X Read Buffer T 4-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 µL/well of Blocker A solution.

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

Step 2: Wash and Add Capture Antibody Solution and Sample

Wash plate 3 times with 300 µL/well of PBS-T.

Add 25 µL/well of 1X capture antibody solution.

Add 25 µL/well of sample (standards, controls, or unknowns).

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

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with 300 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

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

Step 4: Wash and Read Plate

Wash plate 3 times with 300 µL/well of PBS-T.

Add 150 µL/well of 1X Read Buffer T.

Analyze plate on SECTOR Imager.

Plate Diagrams