U-PLEX® Metabolic Group 1 (rat)

Singleplex Assays

MSD U-PLEX Platform

U-PLEX Metabolic Group 1 (rat) Singleplex Assays

For use with serum, EDTA plasma, and cell culture supernatants.

This product insert should 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

Contact Information

MSD Customer Service

Phone: 1-240-314-2795
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-240-314-2798
Fax: 1-240-632-2219 Attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

The U-PLEX Metabolic Group 1 (rat) contains 12 analytes. A complete list of U-PLEX assays can be found at *www.mesoscale.com/en/products_and_services/assay_kits/u-plex_gateway*.

There iis a datasheet available for each assay in the U-PLEX portfolio. A representative data set is presented in those datasheets. The datasheets are available at *www.mesoscale.com/support/product_information*.

Principle of the Assay

Singleplex assays are supplied on either 96-well or 384-well Streptavidin plates (Figure 1). These plates provide high sensitivity and consistent performance. GOLD-branded plates also deliver excellent inter- and intra-lot uniformity.

Each singleplex assay is supplied with a biotinylated capture antibody that binds to streptavidin on the plate surface. Analytes in the sample bind to the capture antibody. Detection antibodies conjugated with electrochemiluminescent labels (MSD GOLD SULFO TAG™) bind to the analytes to complete the sandwich immunoassay. Once the immunoassay is complete, the plate is loaded into an MSD instrument where a voltage applied to the plate causes the captured labels to emit light. The instrument measures the intensity of emitted light (which is proportional to the amount of analyte present in the sample) and provides a quantitative measure of each analyte in the sample.

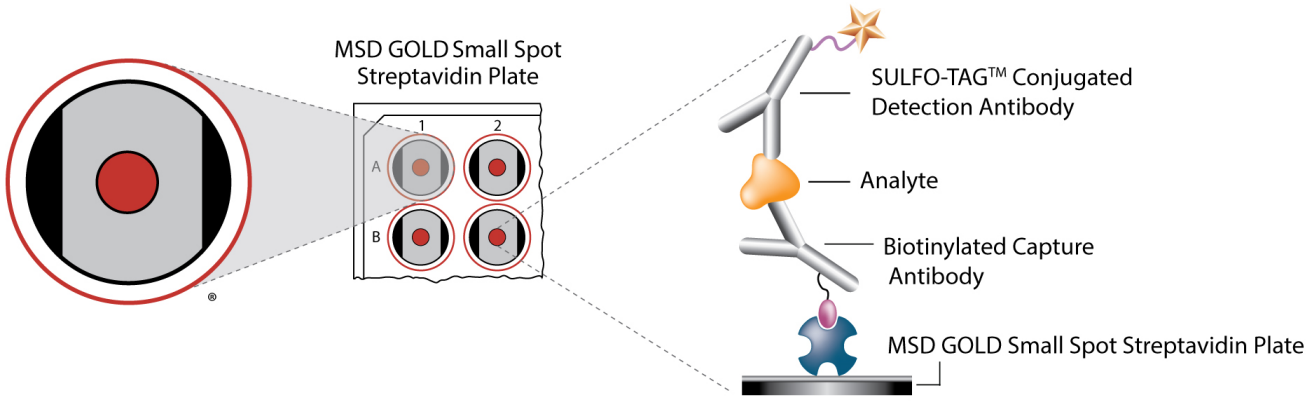

Figure 1. *A U-PLEX singleplex assay on a streptavidin plate.*

Components

Table 1 lists the components provided with U-PLEX Metabolic Group 1 (rat) Singleplex Assays. U-PLEX singleplex assays are available with either SECTOR™ or QuickPlex 96-well plates.

Note: The U-PLEX Rat C-Peptide Assay uses a U-PLEX 2-Assay plate. Use Protocol 2 for this assay.

U-PLEX Singleplex Assays are also available with 384-well SECTOR plates. See Appendix B for details.

Table 1. Reagents that are supplied with all U-PLEX Metabolic Group 1 (rat) 96-well Singleplex Assays

Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
				1 plate	5 plates	25 plates	
MSD GOLD 96-Well Small Spot Streptavidin SECTOR Plate	2–8 °C	L45SA-1	—	1 plate	5 plates	25 plates	96-well plate, foil sealed, with desiccant.
MSD GOLD 96-Well Small Spot Streptavidin QuickPlex Plate		L4BSA-1					96-well plate, foil sealed, with desiccant.
U-PLEX 96-Well 2-Assay 10-Spot SECTOR Plate^		N05227A-1					96-well plate, foil sealed, with desiccant.
U-PLEX 96-Well 2-Assay 10-Spot QuickPlex Ultra Plate^		N04227A-1					
Diluent 100*	2–8 °C*	R50AA-4	50 mL	1 bottle	1 bottle	5 bottles	Diluent for capture antibody
Diluent 13	≤–10 °C	R55BB-4	10 mL	1 bottle	—	—	Diluent for samples and calibrator
		R55BB-3	50 mL	—	1 bottle	5 bottles	
Diluent 11	≤–10 °C	R55BA-5	10 mL	1 bottle	—	—	Diluent for detection antibody
		R55BA-3	50 mL	—	1 bottle	5 bottles	
U-PLEX Linker Set, 1-Assay^	2–8 °C	E2226-2	300 µL	1 vial	—	—	Couples capture antibody to specific spot on plate
Stop Solution^	2–8 °C	R50AO-1	40 mL	1 bottle	1 bottle	5 bottles	Buffer to stop Linker-antibody coupling reaction
MSD GOLD Read Buffer B	RT	E2226-3	1.8 mL	—	1 vial	5 vials	Buffer to catalyze the electrochemiluminescent reaction
		R60AM-2	90 mL	—	1 bottle	5 bottles	

RT = room temperature
Dash (—) = not applicable
*Aliquot and freeze after opening to prevent contamination.
^C-Peptide Assay ONLY

Assay-Specific Reagents

U-PLEX Antibody Set

You will receive a U-PLEX Antibody Set containing a biotinylated capture antibody and a SULFO-TAG conjugated detection antibody (Table 2).

Table 2. Contents of U-PLEX Antibody Set

Name	Storage	Size	Quantity Supplied			Description
			1 Plate	5 Plates	25 Plates	
U-PLEX Analyte-Specific Antibody Set	2–8 °C	1-Plate	1	—	—	Set containing biotinylated capture antibody and SULFO-TAG conjugated detection antibody
		5-Plate	—	1	5	

Dash (—) = not applicable

U-PLEX Calibrators

Metabolic Group 1 calibrators (Table 3) are lyophilized.

Individual analyte concentrations are provided in the lot-specific certificates of analysis (COA). Assays include one vial of the appropriate Calibrator for each assay plate.

Table 3. Analytes included in the Calibrator blends available for U-PLEX Metabolic Group 1 (rat)

Name	Storage	Catalog No.	Analytes
Calibrator 18	2–8 °C	C0292-2	BDNF, C-Peptide, FGF-21, Ghrelin, GLP-1 (inactive), GLP-1 (total), PYY (total)
Calibrator 19	2–8 °C	C0293-2	Glucagon, Insulin, Leptin
Ghrelin (active) Calibrator	2–8 °C	C016K-2	Ghrelin (active)
GLP-1 (active) Calibrator	2–8 °C	C016L-2	GLP-1 (active)

Instrument Compatibility

MSD offers U-PLEX assays designed for use on specific instrument platforms (Table 4).

Table 4. Instrument compatibility

Instrument	Assays on 96-well SECTOR plates	Assays on 96-well QuickPlex® plates	Assays on 384-well SECTOR plates
MESO QuickPlex Q 60MM	—	Y	—
MESO® QuickPlex SQ 120	Y	—	—
MESO QuickPlex® SQ 120MM	Y	—	—
MESO SECTOR S 600MM	Y	—	Y
MESO SECTOR® S 600	Y	—	Y

Y = compatible
Dash (—) = not applicable

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation.
- ❑ Polypropylene tubes for preparing dilutions.
- ❑ Liquid-handling equipment suitable for dispensing 10 to 150 µL/well into a 96-well or 384-well microtiter plate.
- ❑ Plate-washing equipment: automated plate washer or multichannel pipette.
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm (1,500 rpm for 384-well plates).
- ❑ MSD Wash Buffer (20X, 100 mL, catalog number R61AA-1) for plate washing. The standard protocol uses a minimum of 200 mL for a 96-well plate and 415 mL for a 384-well plate. Automated plate washers may need overage added to these volumes.
- ❑ DPP-IV Inhibitor. Formulate and store as recommended by the manufacturer.
- ❑ MSD Block D-R (catalog number R93BR).
- ❑ Adhesive plate seals.
- ❑ Deionized water.
- ❑ Vortex mixer.
- ❑ MSD Diluent 100 (50 mL, catalog number R50AA-4) may be used to dilute samples that need more than 10-fold dilution.

Safety

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

Additional product-specific safety information is available in the applicable safety data sheet (SDS), which can be obtained from MSD Customer Service or at the www.mesoscale.com® website.

Assay Protocol (96-well plates)

Please read the entire detailed Reagent Preparation instructions and the Best Practices (Appendix A) before starting work.

STEP 1: Coat Plates (skip Step 1 if running C-Peptide; see "Prepare U-PLEX Plate" below)

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X Wash Buffer.
- ❑ Add 200 µL of biotinylated capture antibody to 3.3 mL of Diluent 100. Mix by vortexing.
- ❑ Add 25 µL of the biotinylated antibody solution to each well of the provided 96-well MSD plate. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and shake for 1 hour at room temperature.
- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer. The plate is now coated and ready for use.

STEP 2: Add Samples and Calibrators

- ❑ Add 50 µL of prepared Calibrator Standard or sample to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X Wash Buffer.
- ❑ Add 50 µL of detection antibody solution (see Preparation below) to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

STEP 4: Wash and Read

- ❑ Wash the plate 3 times with at least 150 µL/well of 1X Wash Buffer.
- ❑ Add 150 µL of MSD GOLD Read Buffer B to each well. Analyze the plate on an MSD instrument. Incubation in Read Buffer is not required before reading the plate.

Important: The BDNF and Insulin assays in particular will experience a time-dependent decrease in signal upon prolonged incubation in Read Buffer. It is recommended that an MSD instrument be prepared to read a plate before adding Read Buffer for these assays.

Reagent Preparation

Important: Upon the first thaw of diluents, aliquot them into suitable volumes before refreezing.

Dilute Samples

Dilute samples two-fold using Metabolic Assay Working Solution. The dilution factor may need to be optimized for the given sample type. Consult MSD technical support if assistance or additional information is required.

Prepare Metabolic Assay Working Solution

This Solution is used for preparing the calibrator, controls, and diluting the samples.

For one plate, combine the following in a 15 mL tube:

- ❑ 6,965 µL of Assay Diluent
- ❑ 35 µL of aprotinin

Notes:

- Addition of a DPP-IV inhibitor (not provided) to a final concentration of 0.1 mM in the Metabolic Assay Working Solution is strongly recommended. A DPP-IV inhibitor will limit enzymatic action of DPP-IV present in serum/plasma and provides the most accurate measurement of some metabolic analytes.
- **Ghrelin (active) assay only**: Addition of Halt Protease Inhibitor Cocktail, EDTA-Free (Thermo Fisher Scientific, Catalog No. 87785) to a final concentration of 1X in the Metabolic Assay Working Solution is strongly recommended.

Important: Add protease inhibitors immediately before use. Keep the Metabolic Assay Working Solution on ice. Do not freeze the Metabolic Assay Working Solution for later use.

Prepare Detection Antibody Solution

The detection antibody is provided as a 100X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately before use.

For one plate, combine:

- ❑ 60 µL of the supplied 100X detection antibody
- ❑ 5,940 µL of Antibody Diluent

Note, PYY (total) assay only: Addition of MSD Blocker D-R (Catalog No. R93BR, stock concentration 10%) to a final concentration of 0.3% in the Detection Antibody Solution is strongly recommended (180 µL MSD Blocker D-R to a total 6 mL of Detection Antibody Solution). The presence of MSD Blocker D-R does not affect sample quantitation in other assays.

Prepare Calibration Standards

Reconstitution

Bring each Calibrator vial to room temperature (see Figure 2; Table 5). Reconstitute lyophilized Calibrators by adding 250 µL of Metabolic Assay Working Solution to the glass vial. This will result in a 10X concentrated stock of the Calibrator. Invert the reconstituted Calibrator at least 3 times. Do not vortex at this point. Let the reconstituted solution equilibrate at room temperature for 15–30 minutes and then vortex briefly. The Calibrator is now ready for use.

Dilutions

The following instructions are for the preparation of seven Calibrator Standard solutions plus a Zero Calibrator Standard for use in an 8-point standard curve.

Important: Change pipette tips and vortex calibrators after each dilution step. Calibrators are typically run in duplicate. There is sufficient volume of each dilution to run up to six replicates using this process.

- ❑ Prepare Calibrator Standard 1 by adding 25 µL of the reconstituted Calibrator to 225 µL of Metabolic Assay Working Solution. Mix by vortexing.
- ❑ For Calibrator Standard 2, add 75 µL of Calibrator Standard 1 to 225 µL of Metabolic Assay Working Solution.
- ❑ Repeat 4-fold serial dilutions to generate seven Calibrator Standards. Mix by vortexing between each serial dilution.
- ❑ Use Metabolic Assay Working Solution as Calibrator Standard 8 (zero Calibrator).

Note: For the lot-specific concentration of Calibrators in the blend, refer to the COA supplied with the assay. You can also find a copy of the COA at www.mesoscale.com.

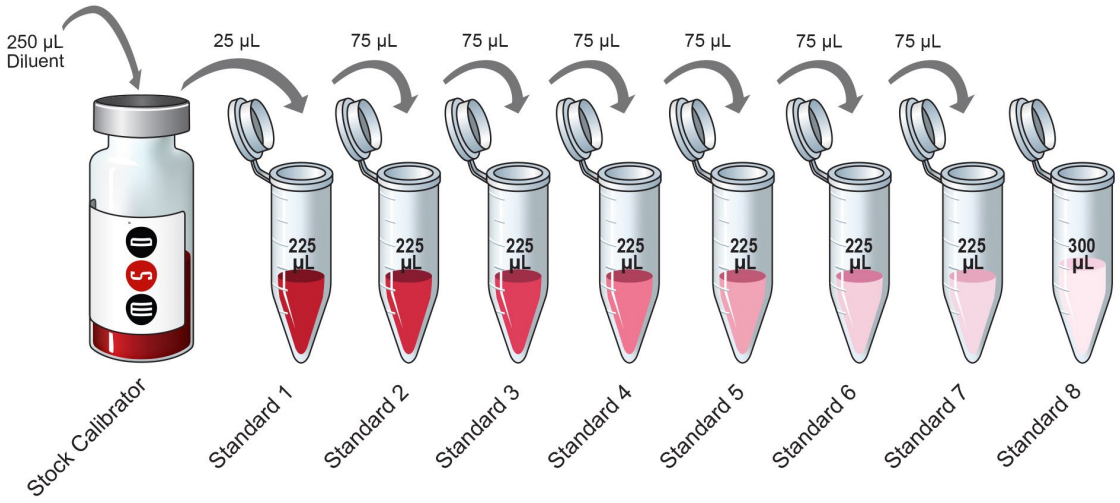

Figure 2. Dilution schema for U-PLEX calibrator standards for singleplex assays.

Table 5. Serial dilution to generate the standard curve

Calibrator Standard No.	Tube No.	Source of Calibrator	Volume of Reconstituted Calibrator (µL)	Metabolic Assay Working Solution (µL)	Total Volume (µL)
1	1	Stock Calibrator vial	25	225	250
2	2	From tube 1	75	225	300
3	3	From tube 2	75	225	300
4	4	From tube 3	75	225	300
5	5	From tube 4	75	225	300
6	6	From tube 5	75	225	300
7	7	From tube 6	75	225	300
8 (zero Calibrator)	8	—	0	300	300

Dash (—) = not applicable

Wash Buffer

Prepare a 1X working solution of MSD Wash Buffer (20X, 100 mL, catalog number R61AA-1) by diluting the 20X stock with deionized water. 1X MSD Wash Buffer can be stored at room temperature for up to two weeks. MSD Wash Buffer (20X, 100 mL, catalog number R61AA-1) is ordered separately.

Read Buffer

MSD provides MSD GOLD Read Buffer B ready for use. Do not dilute.

Prepare U-PLEX Plate (C-Peptide Assay only)

The preparation of a U-PLEX plate involves coating the provided plate with the Linker-coupled capture antibody.

This section describes the preparation of a coating solution for one plate. The volumes can be adjusted depending on the number of plates or wells, but the ratios of the reagents should remain the same (Table 7).

STEP 1: Create U-PLEX Linker-Coupled Antibody Solution

- ❑ Couple the biotinylated capture antibody to the Linker provided.
- ❑ Add 200 µL of the biotinylated antibody to 300 µL of the Linker. Mix by vortexing. Incubate at room temperature for 30 minutes. Do not shake.

Note: To remove liquid from the cap, briefly centrifuge the Linker vial and open the cap gently.

- ❑ Add 200 µL of Stop Solution, then mix by vortexing. Incubate at room temperature for 30 minutes.

Note: At the end of step 1, the U-PLEX Linker-coupled antibody solution is at 10X the coating concentration and can be stored at 2–8 °C. Do not store for more than 7 days.

Adjust the volumes for multiple plates (see Table 5). The volumetric ratio of Linker:antibody:Stop Solution is 3:2:2.

STEP 2: Prepare the Coating Solution for 96-well Plates

- ❑ Combine 600 µL of the U-PLEX Linker-coupled antibody solution with 5.4 mL of Stop Solution to bring the solution up to 6 mL. This will result in a final 1X concentration. Mix by vortexing.

Note: At the end of Step 2, the U-PLEX coating solution is at 1X and can be stored at 2–8 °C. Do not store for more than **7 days.**

STEP 3: Coat U-PLEX 96-well Plates

- ❑ Add 50 µL of the coating solution to each well. Seal the plate with an adhesive plate seal and incubate at room temperature while shaking for 1 hour.
- ❑ Wash the plate 3 times with at least 150 µL/well of 1X MSD Wash Buffer.

Note: The plate is now coated and ready for use. Sealed plates may be stored in the original pouch for up to 7 days at 2–8 °C.

The recommended volumes of Linker, biotinylated capture antibody, and Stop Solution for coating one or multiple U-PLEX plates are provided below in Table 7. If using a partial plate, adjust the volumes used proportionally.

Table 7. Amount of each component required for U-PLEX coating solution per plate

No. of Plates	Individual Linker (µL)	Individual Biotinylated Antibody (µL)	Stop Solution (µL)
1	300	200	200
2	600	400	400
3	900	600	600
4	1,200	800	800
5	1,500	1,000	1,000
N	300 × N	200 × N	200 × N

Appendix A

U-PLEX assays are calibrated against a reference Calibrator generated at MSD.

MSD reference Calibrators for the following analytes were evaluated against the NIBSC/WHO International Standards; the ratios of NIBSC/WHO standard relative to MSD Calibrator are shown in the table below. To convert MSD concentrations to biological activity relative to the WHO International Standard, multiply the MSD concentration by the ratio provided (Table 6).

Table 6. Ratios of NIBSC/WHO Standards relative to MSD Calibrators

Analyte	U-PLEX Calibrator	NIBSC/WHO Cat. No.	NIBSC/WHO Unit	MSD Unit	NIBSC/WHO:MSD
BDNF	Calibrator 18	96/534	U/mL	pg/mL	0.0008
Glucagon	Calibrator 19	69/194	IU/mL	pM	3.46×10^{-9}

Calibrator Conversion Factors

For most assays, the Calibrator concentration provided in the COA is in pg/mL. GLP-1 (active, inactive, total) and Glucagon are provided in pM units, and a factor to convert each to pg/mL is provided in Table 7. Insulin is provided in units/mL, and no conversion factor is available.

Table 7. Calibrator concentration conversion factors.

Assay	pM to pg/mL	
GLP-1 (active)	1 pM	3.30 pg/mL
GLP-1 (inactive)	1 pM	3.09 pg/mL
GLP-1 (total)	1 pM	3.09 pg/mL
Glucagon	1 pM	3.48 pg/mL

Notes regarding the following assays

1. BDNF: The U-PLEX Rat BDNF Assay has been anchored to NIBSC/WHO standard 96/534. Please see Table 7 for conversion factors.
2. Insulin, Leptin, C-Peptide, and PYY (total): The U-PLEX Rat Insulin, Leptin, C-Peptide, and PYY (total) Assays use similar reagents to our previous assays but with different formats and protocols. Each of the U-PLEX Calibrators has been anchored to an internal standard for sustainability. We recommend that users transitioning from previous MSD assays carry out a standard concordance study.
3. GLP-1 (active) and GLP-1 (total): The U-PLEX GLP-1 (active) and GLP-1 (total) Assays have been developed with better sensitivity and improved specificity compared to our previous GLP-1 assays. The U-PLEX GLP-1 (total) Assay detects both active and inactive fragments of GLP-1 equivalently and enables improved quantitation of total GLP-1 in samples. The Calibrators have been anchored to an internal standard for sustainability. We recommend that users transitioning from previous MSD assays carry out a standard concordance study.
4. Glucagon: The U-PLEX Glucagon Assay has been anchored to NIBSC/WHO standard 69/194. Please see Table 7 for conversion factors. Compared to previous versions of the assay, the U-PLEX Glucagon Assay has significantly improved sensitivity and dynamic range and enables measurement of native levels of glucagon in 100% of samples collected in P800 tubes. The measured concentrations of samples may not correlate between the U-PLEX Glucagon Assay and

previous MSD glucagon assays. We recommend that users transitioning from previous MSD glucagon assays carry out a standard concordance study.

Alternative Assay Protocols

The suggestions below may be useful for simplifying the protocol.

- ❑ **Alternate Protocol 1, Co-incubation:** Co-incubating samples and detection antibody solution may improve the sensitivity for some assays. Note that the use of the co-incubation protocol may result in sample concentrations that vary from concentrations obtained with the standard protocol. If this protocol is chosen, we recommend that this protocol be used for the entirety of the research project.
- ❑ **Alternate Protocol 2, Reduced Wash:** For cell culture supernatants, you may simplify the protocol by eliminating one of the washes in each step.

Best Practices

- Equilibrate all assay components to room temperature before use. Mix well. Bring plates to room temperature before opening the packet.
- Avoid bubbles at each stage of reagent addition because they can lead to variable results. This is very important when adding Read Buffer at the final step prior to reading the plate.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm (1,000 to 1,500 rpm for 384-well plates) depending on the shaker design and orbit. Keep the shaking speed and model the same for long-term studies.
- Tap the plate on a paper towel after washing to ensure the removal of residual fluid.
- Avoid excessive drying of the plate during washing steps, especially if working inside a laminar flow hood or another high-airflow environment. Cover the plate with a new plate seal immediately after washing to protect it from airflow, and add solutions to the plate as soon as possible.
- Use a new adhesive plate seal for all incubation steps. Avoid re-using plate seals.
- Dispense reagents and wash fluids at the side of the well towards the bottom corner.
- Remove the plate seal before reading the plate in the instrument.
- Keep time intervals consistent between the addition of Read Buffer and reading the plate to improve inter-plate precision. Prepare an MSD instrument before adding Read Buffer.
- Do not shake the plate after adding Read Buffer.
- Do not obscure or damage the plate barcode; it is required for the plate reader.
- Only use the reagents provided with this kit.
- Use reconstituted or thawed Calibrators immediately. If storage is necessary, divide into suitably sized aliquots, and store immediately at ≤–70 °C.

Appendix B

Components for 384-well plates

Table 6: *Reagents that are supplied with all U-PLEX Metabolic Group 1 (rat) 384-well Singleplex Assays*

Reagent	Storage	Catalog No.	Size	Quantity Supplied		Description
				5 Plates	25 Plates	
MSD 384-well Streptavidin SECTOR Plate	2–8 °C	L21SA-1	—	5 plates	25 plates	384-well plate, foil sealed, with desiccant
U-PLEX 384-Well 2-Assay 4-Spot SECTOR Plate^		N35547A-1				
U-PLEX Linker Set, 1-Assay^	2–8 °C	E2226-3	1.8 mL	1 vial	5 vials	Couples capture antibody to specific spot on plate
Diluent 100*	2–8 °C	R50AA-4	50 mL	2 bottles	10 bottles	Diluent for biotinylated capture antibody and sample dilution
Diluent 13	≤–10 °C	R55BB-3	50 mL	2 bottles	10 bottles	Diluent for samples and Calibrators
Diluent 11	≤–10 °C	R55BA-3	50 mL	2 bottles	10 bottles	Diluent for detection antibody
Stop Solution^	2–8 °C	R50AO-1	40 mL	2 bottles	10 bottles	Buffer to stop Linker-antibody coupling reaction
MSD GOLD Read Buffer B	RT	R60AM-2	90 mL	1 bottle	5 bottles	Buffer to catalyze the electrochemiluminescent reaction

Dash (—) = not applicable
RT = room temperature
*Aliquot and freeze after opening to prevent contamination.
^C-Peptide Assay ONLY

Reagent Preparation for 384-well Plates

Important: Upon the first thaw, aliquot diluents into suitably sized aliquots before refreezing.

Prepare Metabolic Assay Working Solution

This Solution is used for preparing the calibrator, controls, and diluting the samples.

For one 384-well plate, combine the following in a 15 mL tube:

- ❑ 13.93 mL of Assay Diluent
- ❑ 70 µL of aprotinin

Notes:

- Addition of a DPP-IV inhibitor (not provided) to a final concentration of 0.1 mM in the Metabolic Assay Working Solution is strongly recommended. A DPP-IV inhibitor will limit enzymatic action of DPP-IV present in serum/plasma and provides the most accurate measurement of some metabolic analytes.
- Ghrelin (active) assay only: Addition of Halt Protease Inhibitor Cocktail, EDTA-Free (Thermo Fisher Scientific, Catalog No. 87785) to a final concentration of 1X in the Metabolic Assay Working Solution is strongly recommended.

Important: Add protease inhibitors immediately before use. Keep the Metabolic Assay Working Solution on ice. Do not freeze the Metabolic Assay Working Solution for later use.

Coat 384-well Plate (see below for C-Peptide

- ❑ Add 240 µL of biotinylated capture antibody to 11.76 mL of Diluent 100. Mix by vortexing.

- ❑ Add 25 µL of the above solution to each well of the provided plate. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and incubate with shaking at room temperature for 2 hours.
- ❑ Wash the plate 3 times with 80 µL/well of 1X MSD Wash Buffer. The plate is now coated and ready for use. Plates may be sealed and stored overnight at 4 °C.

Plate Preparation for C-Peptide in 384-well U-PLEX Plates

Important: Upon the first thaw, aliquot diluents into suitably sized aliquots before refreezing.

STEP 1: Create U-PLEX Linker-Coupled Antibody Solution

Below are the steps to couple the linker with the capture antibody.

- ❑ Add 200 µL of the biotinylated antibody to 300 µL of the Linker. Mix by vortexing. Incubate at room temperature for 30 minutes. Do not shake.

Notes: To remove liquid from the cap, briefly centrifuge the Linker vial and open the cap gently.

- ❑ Add 200 µL of Stop Solution, then mix by vortexing. Incubate at room temperature for 30 minutes.

Note: At the end of step 1, the U-PLEX Linker-coupled antibody solution is at 10X the coating concentration and can be stored at 2–8 °C. Do not store for more than 7 days.

Adjust the volumes for multiple plates (see Table 7). The volumetric ratio of Linker:antibody:Stop Solution is 3:2:2.

STEP 2: Prepare the Coating Solution for C-Peptide in 384-well Plates

- ❑ Combine 600 µL of the U-PLEX Linker-coupled antibody solution with 11.4 mL of with Stop Solution. Mix by vortexing.

Note: At the end of Step 2, the U-PLEX coating solution is at 0.5X and can be stored at 2–8 °C. Do not store for more than 7 days.

STEP 3: Coat U-PLEX 384-well Plates

- ❑ Wash the plate 3 times with 80 µL/well of 1X Wash Buffer.
- ❑ Add 25 µL of the 0.5X coating solution to each well. Seal the plate with an adhesive plate seal and shake for 4 hours at room temperature.
- ❑ Wash the plate 3 times with 80 µL/well of 1X Wash Buffer.

Note: The plate is now coated and ready for use. Sealed plates may be stored in the original pouch for up to 7 days at 2–8 °C.

Prepare Detection Antibody Solution

The detection antibody is provided as a 100X stock solution. The working solution is 0.5X. Prepare the detection antibody solution immediately before use.

For one plate, combine:

- ❑ 60 µL of the supplied 100X detection antibody
- ❑ 11.94 mL of Antibody Diluent

Note, PYY (total) assay only: Addition of MSD Blocker D-R (Catalog No. R93BR, stock concentration 10%) to a final concentration of 0.3% in the Detection Antibody Solution is strongly recommended (360 µL MSD Blocker D-R to a total 12 mL of Detection Antibody Solution). The presence of MSD Blocker D-R does not affect sample quantitation in other assays.

Assay Protocol (384-well plates)

Important: Please read the entire detailed Reagent Preparation instructions and the Best Practices (Appendix A) before starting work.

STEP 1: Coat Plates (skip Step 1 if running C-Peptide)

- ❑ Wash the plate 3 times with at least 80 µL/well of 1X Wash Buffer.
- ❑ Add 240 µL of biotinylated capture antibody to 11.76 mL of Diluent 100. Mix by vortexing.
- ❑ Add 25 µL of the biotinylated antibody solution to each well of the provided 384-well MSD plate. Tap the plate gently on all sides. Seal the plate with an adhesive plate seal and shake for 1 hour at room temperature.
- ❑ Wash the plate 3 times with at least 80 µL/well of 1X MSD Wash Buffer. The plate is now coated and ready for use.

STEP 2: Add Samples and Calibrators

- ❑ Wash the plate 3 times with 80 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL of the prepared Calibrator Standard or sample to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 80 µL/well of 1X MSD Wash Buffer.
- ❑ Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal. Incubate at room temperature with shaking for 2 hours.

STEP 4: Wash and Read

- ❑ Wash the plate 3 times with 80 µL/well of 1X MSD Wash Buffer.
- ❑ Add 40 µL of MSD GOLD Read Buffer B to each well. Analyze the plate on an MSD instrument. Incubation in Read Buffer is not required before reading the plate.

Important: The BDNF and Insulin assays in particular will experience a time-dependent decrease in signal upon prolonged incubation in Read Buffer. It is recommended that an MSD instrument be prepared to read a plate before adding Read Buffer for these assays.

Alternative Assay Protocols

The suggestions below may be useful for simplifying the protocol.

- ❑ **Alternate Protocol, Shortened Incubation:** Some 384-well assays may achieve acceptable performance with shorter incubations. Consider reducing the incubation time of samples in the plate and the detection antibody incubation time.

Plate Diagrams

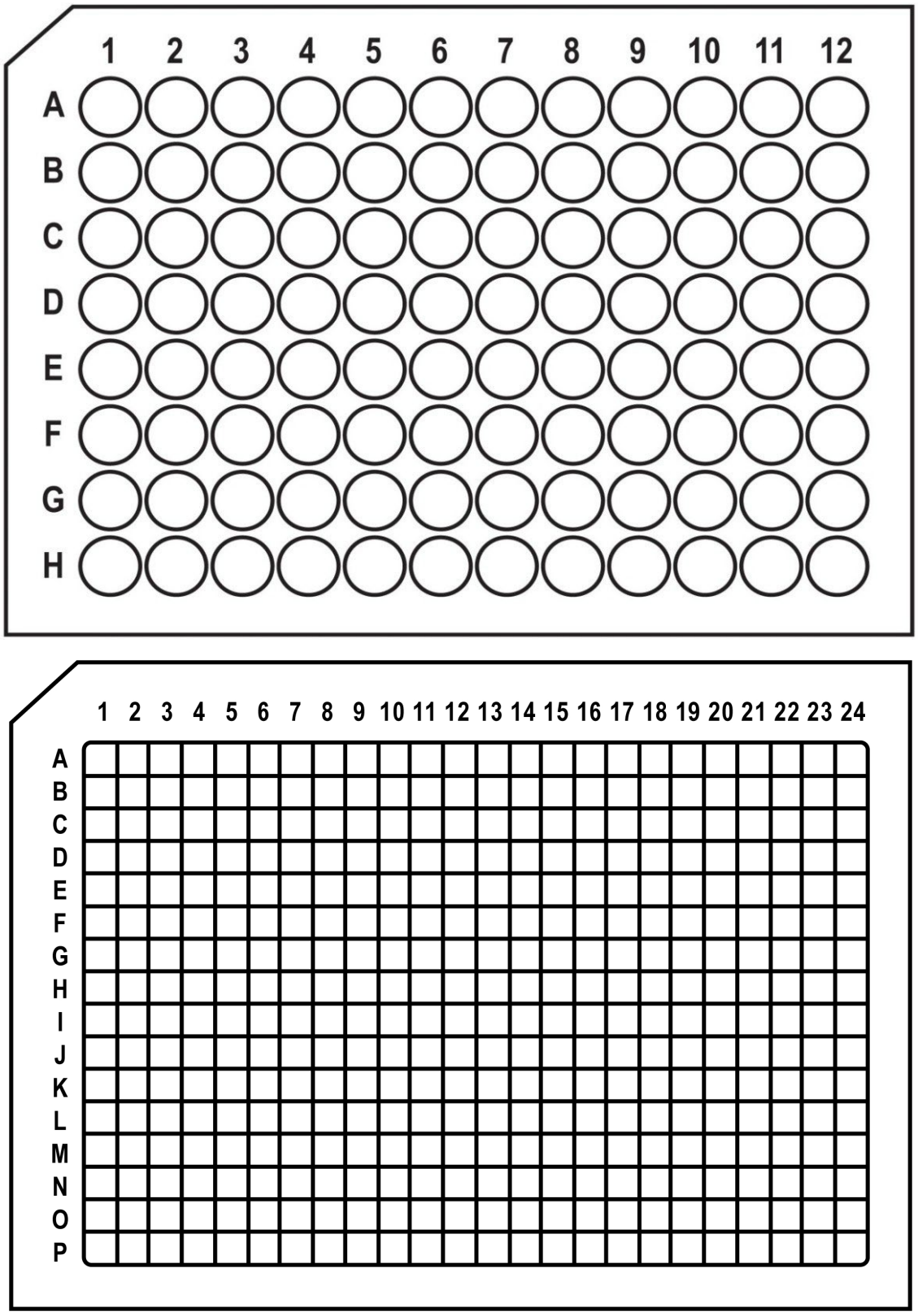

Figure 3. *Plate diagrams. Similar plate layouts can be created in Excel and in the DISCOVERY WORKBENCH® software.*