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U-PLEX® Metabolic Group 1 (mouse) Multiplex Assays

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Abstract

The U-PLEX platform allows the creation of custom multiplex assays for any combination of analytes in the same Group.
This product insert is for U-PLEX Combos and multiplex assays that contain any number of the 58 assays in the U-PLEX Metabolic Group 1 (mouse). Using open spots, custom combinations can include R-PLEX® Antibody Sets or your antibodies for other analytes.
Representative data for each U-PLEX assay is presented in the product-specific datasheets available at the **www.mesoscale.com**® website. The performance of MSD assays may vary when tested in combination. The data presented in the datasheets do not represent the product specifications

Guidelines

Principle of the Assay

Biotinylated capture antibodies are coupled to U-PLEX Linkers, which self-assemble onto unique spots on the U-PLEX plate. Analytes in the sample bind to the capture reagents. Detection antibodies conjugated with electrochemiluminescent labels (MSD GOLD™ SULFO-TAG) bind to the analytes to complete the sandwich immunoassay (Figure 1). Once the sandwich immunoassay is complete, the U-PLEX plate is loaded into an MSD instrument where a voltage applied to the plate electrodes 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.

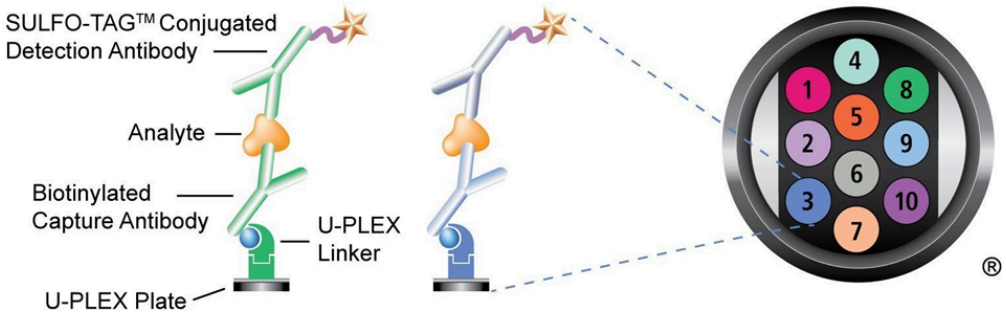

Figure 1. U-PLEX multiplex immunoassay on a U-PLEX 96-well 10-Assay Plate. U-PLEX 384-well 4-Assay plates are similar.

Spot Maps

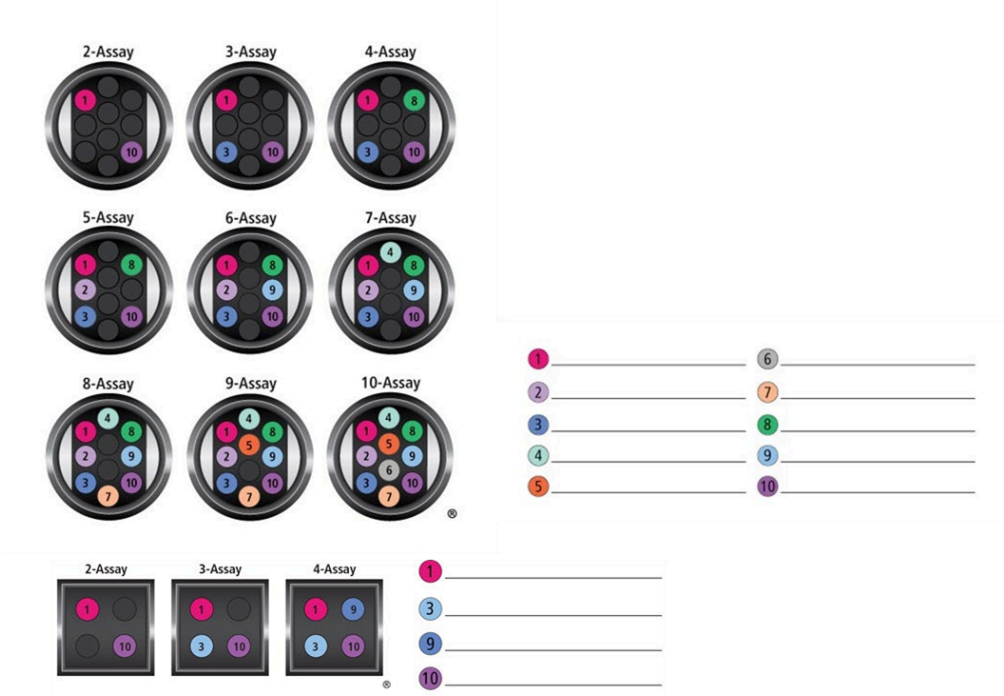

Figure 5. Spot maps. (top) 96-well plate, (bottom) 384-well plate.

Materials

Components

Tables 1, 2, and 3 list the components provided with multiplex U-PLEX Metabolic Group 1 (mouse) Assays. You will only receive components relevant to the assays that you order.

	A	B	C	D	E	F	G	H
	Reagent	Storage	Catalog No.	Size	Quantity Supplied			Description
					1 Plate	5 Plates	25 Plates	
	Diluent 13	≤-10 °C	R56BB-4	10 mL	1 bottle	—	—	Diluent for samples and calibrators
			R56BB-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	
	Aprotinin (200,000 KIU/mL)	2–8 °C	R93AD-1	50 µL	1 vial	—	—	200X concentrated solution
			R93AD-2	250 µL	—	1 vial	5 vials	
	Stop Solution	2–8 °C	R50AO-1	40 mL	1 bottle	1 bottle	5 bottles	Biotin-containing buffer to stop Linker-antibody coupling reaction
	MSD GOLD Read Buffer B	RT	R60AM-1	18 mL	1 bottle	—	—	Buffer to catalyze the electrochemiluminescent

	A	B	C	D	E	F	G	H
			R60AM-2	90 mL	—	1 bottle	5 bottles	reaction

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

RT = room temperature
Dash (—) = not applicable

Assay-Specific Reagents

U-PLEX plates are provided in a sealed foil pouch with desiccant. The spots correspond to unique U-PLEX Linkers. The number and layout of the active spots on the plate depend on the plate well density (96 vs 384) and the number of assays to be multiplexed (Figure 2). For example, if 4 assays are to be multiplexed, either a U-PLEX 96-well or 384-well 4-Assay Plate will be provided.

U-PLEX Plates

U-PLEX multiplex assays include plates that are specific to the assay with which they ship. Do not separate plates from the other refrigerated components.

A. 96-well spot maps

B. 384-well spot maps

Figure 2. Spot Maps of the different U-PLEX multiplex plates showing the placement of Linkers within a well. The colored spots represent the active U-PLEX binding spots. The numbering convention for the different spots is maintained in the software visualization tools and in the data files.

Linkers

Each Linker has a biotin-binding domain that couples to the biotinylated capture antibody, as well as a domain that binds to its matching spot on the U-PLEX plate. The Linkers are color-coded and numbered with the spot to which they attach on the plate.
Record which antibody is coupled to each Linker when performing the coupling step (as described in the Reagent Preparation section).

U-PLEX Antibody Sets

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

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

Table 2. Contents of U-PLEX Antibody Sets

Dash (—) = not applicable

U-PLEX Calibrators

Calibrators may be either lyophilized or frozen. Individual analyte concentrations are provided in lot-specific certificates of analysis (COA). Depending on the specific assays requested, one or more of the following Calibrators will be provided (Table 3).

	A	B	C	D
	Name	Storage	Catalog No.	Analytes

	A	B	C	D
	Calibrator 5	2–8 °C	C0065-2	EPO, GM-CSF, IFN-g, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, KC/GRO, TNF-a, VEGF-A
	Calibrator 7	2–8 °C	C0073-2	IL-16, IL-17A, IL-17C, IL-17E/IL-25, IL-21, IL-22, IL-23
	Calibrator 8	2–8 °C	C0074-2	IL-15, IL-17F, IL-31, IL-33, IL-27p28/IL-30
	Calibrator 12	2–8 °C	C0092-2	IL-9, IL-17A/F, IP-10, MCP-1, MIP-1a, MIP-1b, MIP-2, MIP-3a
	Calibrator 16	≤–70 °C	C0295-2	6CKine/CCL21, BAFF, BCA-1/BLC, CD40, IFN-β, MCP-5/CCL12, MDC
	Calibrator 17	2–8 °C	C0296-2	Eotaxin, MMP-9 (total), NGAL/LCN2, RANTES, SDF-1α, TARC, TNF-RI
	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)	2–8 °C	C016K-2	Ghrelin (active)
	GLP-1 (active)	2–8 °C	C016L-2	GLP-1 (active)
	Mouse IFN-**α** Calibrator	2–8 °C	C02W1-2	IFN-α

Table 3. Analytes included in the Calibrators available for U-PLEX Metabolic Group 1 (mouse)

Additional Materials and Equipment

- Appropriately sized tubes for reagent preparation.
- Polypropylene microcentrifuge tubes for preparing dilutions.
- Liquid-handling equipment suitable for dispensing 10 to 150 µL/well into a 96-well microtiter plate.
- Plate-washing equipment: automated plate washer or multichannel pipette.
- Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm (1,000–1,500 for 384-well plates).
-

Wash Buffer (20x) **MESO SCALE DIAGNOSTICS, LLC. Catalog #**R61AA-1 for plate washing. The standard protocol uses a minimum of 415 mL of 1X Wash Buffer for a 384-well plate and 130 mL for a 96-well plate. Automated plate washers may need overage added to these volumes.

- Adhesive plate seals.
- Deionized water.
- A dipeptidyl peptidase IV (DPP-IV) inhibitor (such as diprotin A) is recommended for certain assays.
-

MSD Blocker D-R **MESO SCALE DIAGNOSTICS, LLC. Catalog #**R93BR is recommended for certain assays.

-

Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X) **Thermo Fisher Catalog #**87785 . Recommended for Ghrelin (active) assay. Use as recommended by the manufacturer.

- Vortex mixer.

Note

If including Open Spots, you will also need:

- MSD GOLD SULFO-TAG NHS-Ester (catalog no. R91AO-1) for conjugating detection reagents or SULFO-TAG conjugated antispecies antibodies for use as reporters with unconjugated detection antibodies.
- Sulfo-NHS-LC-Biotin for biotinylating the capture reagents (e.g., EZ-Link Sulfo-NHS-LC-Biotin, Thermo Fisher Scientific, catalog no. 21327, or equivalent).

MSD GOLD SULFO-TAG NHS-Ester **MESO SCALE DIAGNOSTICS, LLC. Catalog #**R91AO-1

- Zeba Desalting Columns (Thermo Fisher Scientific, catalog numbers 87766-87773).

Zeba™ Spin Desalting Columns, 40K MWCO, 0.5 mL **Thermo Fisher Catalog #**87766

Zeba™ Spin Desalting Columns, 40K MWCO, 0.5 mL **Thermo Fisher Catalog #**87767

Zeba™ Spin Desalting Columns, 40K MWCO, 2 mL **Thermo Fisher Catalog #**87768

Zeba™ Spin Desalting Columns, 40K MWCO, 2 mL **Thermo Fisher Catalog #**87769

Zeba™ Spin Desalting Columns, 40K MWCO, 5 mL **Thermo Fisher Catalog #**87770

Zeba™ Spin Desalting Columns, 40K MWCO, 5 mL **Thermo Fisher Catalog #**87771

Zeba™ Spin Desalting Columns, 40K MWCO, 10 mL **Thermo Fisher Catalog #**87772

Zeba™ Spin Desalting Columns, 40K MWCO, 10 mL **Thermo Fisher Catalog #**87773

- Coating diluent such as 0.5% bovine serum albumin in PBS, or MSD Diluent 100 for diluting the capture antibody.

Instrument Compatibility

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

	A	B	C
	Instrument	Assays on U-PLEX 96-well SECTOR™ Plate	Assays on U-PLEX 384-well SECTOR Plate
	MESO® QuickPlex SQ 120	Y	—
	MESO QuickPlex® SQ 120MM	Y	—
	MESO SECTOR® S 600	Y	Y
	MESO SECTOR S 600MM	Y	Y

Table 4. Instrument compatibility

Dash (—) = not applicable

Prepare Calibrator Standards

For Lyophilized Calibrators

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

For Liquid Calibrators

Thaw the stock Calibrator(s) and gently mix. Keep on ice. Once thawed, the Calibrator is ready to use.

Note

We recommend that reconstituted or thawed Calibrators be used immediately. If storage is necessary, divide Calibrators into suitably sized aliquots (60 µL aliquots are recommended) and store immediately at ≤−70 °C.

Prepare Calibrator Standard 1 (top of the curve) in a polypropylene tube by mixing and diluting the reconstituted or thawed Calibrator as indicated in Table 6. Mix by vortexing.

	A	B	C	D
	No. of Calibrators Provided	Volume of Reconstituted Calibrator (µL)	Metabolic Assay Working Solution (µL)	Total volume (µL)
	2	25 each	200	250
	3	25 each	175	250
	4	25 each	150	250
	N	25 each	250 - (25 x N)	250

Table 6. Combining Calibrators to generate the Calibrator Standard 1 (top of the curve) level

Prepare the subsequent 6 dilutions for the curve (4-fold serial dilutions) in Metabolic Assay Working Solution (see Figure 4; Table 7). Use Metabolic Assay Working Solution for the Calibrator Standard 8 (zero Calibrator/blank). Mix by vortexing the tubes between each serial dilution.

	A	B	C	D	E	F
	Calibrator Standard No.	Tube No.	Source of Calibrator	Volume of Reconstituted Calibrator (µL)	Metabolic Assay Working Solution (µL)	Total volume (µL)
	1	1	Calibrator Standard 1 (top of curve)	See Table 6		
	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

	A	B	C	D	E	F
	7	7	From tube 6	75	225	300
	8 (zero Calibrator)	8	—	0	300	300

Table 7. Serial dilution to generate the standard curve

Dash (—) = not applicable

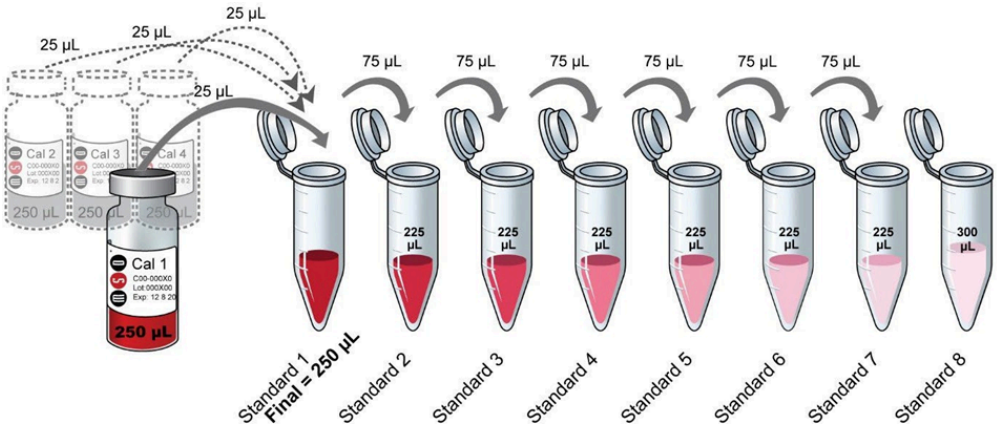

Figure 4. Dilution schema for preparation of Calibrator Standards for U-PLEX Metabolic Group 1 (mouse) Assays.

Sample Collection and Handling

Below are general guidelines for sample collection, storage, and handling for metabolic markers. We strongly suggest following these procedures if working with the active forms of protein analytes. If other methods are used, evaluate sample stability under the selected method as needed.
The assay requires 50 µL/well of the sample (25 µL for 384-well plates). Based on the number of replicates desired, prepare an adequate volume of the sample.
Sample Collection (preferred method): Samples should be collected using the BD P800 Collection and Preservation System, which contains a DPP-IV and other protease inhibitor cocktails (Product Number 366420 or 366421). The alternative collection method described below with K_2EDTA tubes can also be used.

Non-P800 collection method: Collect blood in BD Vacutainer K_2EDTA Tubes (Product Number 367841 or 366643).

Immediately add a DPP-IV inhibitor (0.1 mM final concentration, not provided) and aprotinin (1,000 KIU/mL final concentration) and mix to avoid cleavage/degradation of metabolic peptides.

For BD tubes, process as follows:

θ In a swing-out rotor centrifuge, spin the blood collection tubes as follows;

- For 2 mL tubes —10 minutes at 1,000 × g (2–8 °C).
- For 8.5 and 10 mL tubes—20 minutes at 1,300 × g (2–8 °C).

θ Use the plasma immediately or the samples can be stored at 2–8 °C if used within 3 hours. For future use, aliquot the plasma and freeze in suitably sized aliquots at ≤–70 °C.

For samples other than serum and plasma, add a DPP-IV inhibitor (0.1 mM final concentration, not provided) and aprotinin (1,000 KIU/mL final concentration) and use immediately or freeze at ≤–70 °C.
Samples with hemolysis or significant lipemia may hinder accurate measurements.
Repeated freezing and thawing of samples is not recommended. After thawing, centrifuge samples at 2,000 × g for 3 minutes to remove particulates before use in the assay. If the samples are clear and no particulates are visible, you may not need to centrifuge. Hold on wet ice or 2–8 °C until processed and used in the assay.

Dilute Samples

Depending on the sample set under investigation, dilution may be necessary. At least a two-fold dilution of serum and plasma is recommended to reduce matrix effects. Metabolic Assay Working Solution should be used for sample dilution. The dilution factor for the given sample type may need to be optimized.

Note

For BAFF, the concentrations in normal serum and EDTA plasma may exceed the standard working range of the assays. Refer to the product-specific datasheets for additional information.

Wash Buffer

Prepare a 1X working solution by diluting the 20X stock with deionized water.

Read Buffer

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

Safety warnings

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(s) (SDS), which can be obtained from MSD Customer Service or at **www.mesoscale.com.**

Files

SEARCH

Protocol

NAME

Assay Protocol (96-well plates)

VERSION 1

CREATED BY

Alexandria White
Emory University

OPEN →

Protocol

NAME

Assay Protocol (384-well plates)

VERSION 1

CREATED BY

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