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ProcartaPlex™ Human Liver Toxicity Panel 2 5-Plex

USER GUIDE

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For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0030026 A.0 (30) (English)

Revision	Date	Description
A.0 (30)	2 November 2023	New document for ProcartaPlex™ Human Liver Toxicity Panel 2 5-Plex.

The information in this guide is subject to change without notice.

Contents

1 Product Information

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product Description

The ProcartaPlex™ Human Liver Toxicity Panel 2 5-Plex has been optimized for detection of multiple analytes from cell lysates.

The panel is provided in a ready-to-use format with individual vials of 1X capture and detection reagents that require less pipetting and experimental setup. These reagents are not combinable with simplexes or other panels.

ProcartaPlex™ preconfigured panels are extensively tested for analyte combinability, interference and cross-reactivity to provide the highest level of validation and precision. All ProcartaPlex™ panels are supplied with the necessary reagents to perform the assay.

Table 1 Analytes

B2M	CYP2C19	CYP2D6
CYP2E1	GAPDH	

Contents and storage

Upon receipt, store the kit at 2°C to 8°C. When stored as indicated, all reagents are stable until the expiration date.

Contents	Amount
Standard Mix 20 (lyophilized)	2 each
Standard Mix 21 (lyophilized)	2 each
Biotinylated Detection Antibody Mix (1X)	1 x 3.5 mL
Capture Bead Mix (1X)	1 x 5 mL
Streptavidin-PE (SA-PE) (1X)	1 x 5 mL
Wash Buffer (10X)	1 x 25 mL
Reading Buffer (1X)	1 x 40 mL
Universal Assay Buffer, UAB (1X)	1 x 10 mL
8-Tube Strip	2 each
Flat Bottom 96-well Plate	1 each
Microplate Lid	1 each
Plate Seals	8 each

Retain the lot-specific Certificate of Analysis that contains the product expiration date. The Certificate of Analysis also contains important information such as bead number, analyte names, and highest standard concentration required for the assay setup on the xMAP™ instrument.

Required materials not supplied

Catalog numbers that appear as links open the web pages for those products.

- xMAP™-based instrument
- Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000)
- Cell Extraction Buffer (Cat. No. FNN0011)
- Halt™ Protease Inhibitor Cocktail 100X (Cat. No. 78430; includes a separate vial of EDTA solution)
- Deionized water
- Vortex mixer (for example, Cat. No. 88882010)
- Microcentrifuge
- Adjustable single and multichannel pipettes with disposable tips and low-volume reservoirs (for example, Cat. No. 95128093)
- Beakers, flasks, and cylinders (for preparation of reagents)

- Orbital microplate shaker with at least 1.5 mm or 0.059 inch orbit diameter capable of maintaining a speed of 600 ± 50 rpm (for example, Cat. No. 88882006)
- *Optional*: QuantiGene™ Lysis mixture (Cat. No. QP0522)

Note: Use of rockers or large orbital shakers can cause adverse results.

Procedural guidelines

- Thoroughly read this user guide and the certificate of analysis before using the kit.
- All chemicals should be considered potentially hazardous.
- To avoid cross-contamination, do not invert the assay plate during the assay or allow contents from one well to mix with another well.
- Use a multichannel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.
- This protocol was developed using the Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000). Other washers should be validated by the end user.
- Ensure that the xMAP™ instrument has been properly calibrated and set up before preparing and running the assay.

Workflow

Assay protocol

Prepare antigen standard

Add capture beads

1. Vortex capture beads for 30 sec. Add 50 µL of the capture beads to each well.
2. Remove the liquid, then wash the plate.

Add samples and standards

1. **For cell lysate samples:** Add 25 µL of 1X UAB, then add 25 µL of cell lysate. For background wells, add 50 µL of 1X UAB.
2. Seal the plate and incubate with shaking at room temp for 2 hr.
3. Wash plate two times.

Add detection antibody

1. Add 25 µL of Detection Antibody Mix (1X).
2. Seal the plate and incubate with shaking at room temp for 30 min.
3. Wash plate two times.

Add Streptavidin-PE

1. Add 50 µL of Streptavidin-PE.
2. Seal the plate and incubate with shaking at room temp for 30 min.
3. Wash plate two times.

Resuspend beads

1. Add 120 µL of Reading Buffer.
2. Seal the plate and shake at room temp for 5 min.

Acquire data on xMAP™ system

2 Methods

Prepare the samples

This assay is suitable for cell lysates only.

Prepare the cell lysate sample

Complement the Cell Extraction buffer (Cat. No. FNN0011) with 0.5 M EDTA and Halt™ Protease Inhibitor Cocktail, 100X, (Cat. No. 78430). For example, add 100 µL of 0.5 M EDTA Solution and 100 µL Halt™Protease Inhibitor cocktail, 100X, to 9.8 mL of Cell Extraction buffer.

1. Stimulate cells as desired.

   Note: We recommend at least 50,000 cells/well (96-well plate) are seeded.

2. Remove cell media.
3. Wash 3 times with ice-cold PBS (1X).
4. Add 100 µL of Cell Extraction buffer directly to the pellet. The volume of Cell Extraction buffer depends on the cell number and the level of expression of the target protein.
5. Incubate on ice for 10 minutes.
6. Determine the total protein concentration of the lysate sample by following the manufacturer's protocol of a protein assay kit (e.g. Pierce™ BCA Protein Assay Kits, Cat. No. A55864).

IMPORTANT! Proceed to analysis immediately after lysis, or freeze and store the cell lysates at -80°C. If you want to determine mRNA levels in the same sample, use 30 µL of cell lysate aliquoted into 15 µL QuantiGene Lysis mixture (Cat. No. QP0522) and proceed according to the QuantiGene User manual.

Note: Avoid multiple freeze-thaw cycles of the frozen cell lysates. Thaw completely and mix well before use.

Prepare the reagents

Before starting with the assay protocol, define the plate map. Mark the standard, sample, and background wells to determine the number of wells used (see Appendix A, “Recommended plate layout”).

Prepare 1X Wash Buffer

Bring the Wash Buffer Concentrate (10X) to room temperature and vortex for 15 seconds. Mix 20 mL of the Wash Buffer Concentrate (10X) with 180 mL ddH_2O. Mix gently to avoid foaming. Wash Buffer (1X) can be stored at 2–8°C for up to 6 months.

Note: Additional Wash Buffer Concentrate (200 mL, Cat. No. EPX-66666-001) can be purchased separately for automated plate washers.

Prepare Standard Mix

This kit is supplied with two lyophilized Standard Mixes for generation of standard curves. Two vials of each Standard Mix are provided to permit the user to run the assay twice if running a partial plate.

Note: Change pipette tips after each dilution step and avoid air bubbles.

1. Centrifuge each standard mix stock vial at 2,000 x *g* for 10 seconds.
2. Add 125 µL of diluent to each stock vial.
3. Vortex the vial at high speed for 30 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vial.
4. Incubate on ice for 10 minutes to ensure complete reconstitution.
5. Combine the contents of each of the two Standard Mix vials into one vial.

Prepare 4-fold serial dilution

1. Label the tubes in the 8-Tube Strip: Std1, Std2, Std3, Std4, Std5, Std6 and Std7.
2. Add 200 µL of the reconstituted standard mix into Std1 tube.
3. Add 150 µL of diluent into Std2–Std7 tubes.
4. Transfer 50 µL from Std1 tube into Std2 tube.
5. Mix by pipetting up and down 10 times.
6. Transfer 50 µL of the mixed standards from Std2 tube into Std3 tube using new pipette tip.
7. Mix by pipetting up and down 10 times.
8. Repeat steps 4–7 for tubes Std4–Std7, changing pipette tips between dilution steps.
9. Add 150 µL of diluent to the last tube of the 8-Tube Strip to serve as a background. See Figure 1.

10. Keep tubes on ice until ready to use.

Note: Use the reconstituted standard immediately. The reconstituted standard cannot be stored. Discard unopened standard vials if the entire plate was used in a single experiment.

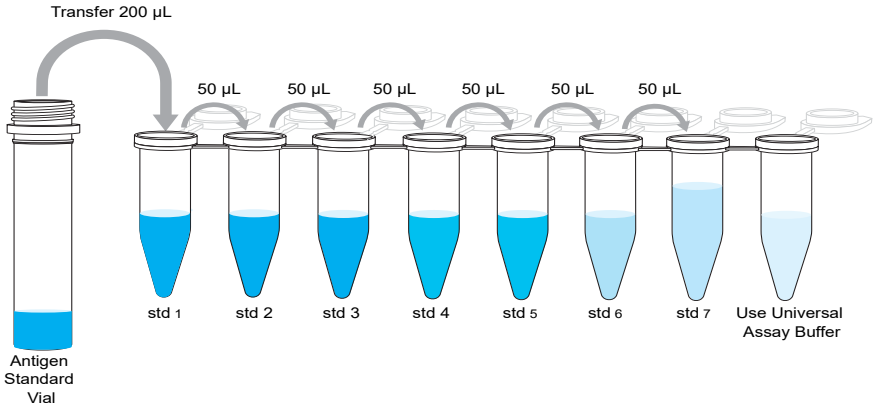

Figure 1 4-fold serial dilution

Assay protocol

1. Add Capture Bead Mix to the plate.
   a. Vortex the 1X Capture Bead Mix vial for 30 seconds at high speed.
   b. Using a multichannel pipette, add 50 µL of the Capture Bead Mix to each well of the plate.
2. Wash beads using a Hand-Held Magnetic Plate Washer.

Note: To avoid loss of beads, secure the plate using the clamps on both sides of the Hand-Held Magnetic Plate Washer during this procedure.

Note: This protocol was developed using the Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000). Other washers should be validated by the end user.

   a. Place the plate on the Hand-Held Magnetic Plate Washer and wait 2 minutes to allow the beads to settle on the bottom of each well.
   b. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.
   c. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.
   d. Add 150 µL of 1X Wash Buffer into each well and wait 30 seconds.
   e. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.

f. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.

g. Remove the plate from the magnet and proceed to step 3.

3. Add samples and standards to the plate.
   a. Add 25 µL of Universal Assay buffer, then add 25 µL of standards or samples. For background wells, add 50 µL of 1X UAB.

   b. Seal the plate using one of the provided Plate Seals and cover with the provided Microplate Lid. Shake at 600 rpm for 2 hours at room temperature.

4. Remove and discard the Plate Seal. Wash the plate following the steps below.
   a. Place the plate on the Hand-Held Magnetic Plate Washer and wait 2 minutes to allow particles to settle on the bottom of each well.

   b. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.

   c. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.

   d. Add 150 µL of 1X Wash Buffer into each well and wait 30 seconds.

   e. Remove the liquid by quickly inverting the washer/plate assembly over a sink or waste container.

   f. Gently blot the inverted washer/plate assembly onto several layers of paper towels or absorbent surface to remove any residual liquid.

   g. Repeat steps 4d-4f once more for a total of two washes.

   h. Remove the plate from the magnet and proceed to the next step.

5. Add Biotinylated detection Antibody Mix to the plate.
   a. Using a multichannel pipette, add 25 µL of the detection antibody solution to each well of the plate. Gently tap the plate to evenly distribute the solution in the wells.

   Note: A narrow trough reservoir for multichannel pipetting is recommended to be used to prevent volume loss.

   b. Seal the plate using a new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.

6. Wash the plate following step 4.

7. Add Streptavidin-PE (SA-PE) to the plate.
   a. Add 50 µL of SA-PE solution to each well.

b. Seal the plate using new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 30 minutes at room temperature.

8. Wash the plate following step 4.

9. Prepare the plate for analysis on an xMAP™ instrument.
   a. Add 120 µL of reading buffer into each well.

   b. Seal the plate using new Plate Seal and cover with the provided Microplate Lid. Shake at 600 rpm for 5 minutes at room temperature.

10. Remove the Plate Seal and run the plate on an xMAP™ instrument.

Note: Alternatively, the sealed plate can be stored overnight at 2-8°C before running on an xMAP™ instrument. Shake the plate at 600 rpm for 5 minutes at room temperature then remove the plate seal and run the plate.

Instrument settings

Follow the recommended guidelines and procedures for calibration and verification of the instrument. Laser-based systems require 30 minutes to warm up prior to use.

Instrument	Acquisition volume	Timeout (optional)	Bead type	DD gate	Reporter gain	Min. bead count
MAGPIX™	50 µL[1]	N/A	N/A	N/A	Standard PMT	50
INTELLIFLEX™	30 µL	40 sec	MagPlex™	7,000–17,000	Standard PMT	50
FLEXMAP 3D™ Luminex™ 100/200™	50 µL	60 sec	MagPlex™	7,500–25,000	Standard PMT	50
Bio-Rad™ Bio-Plex™	50 µL	60 sec	MagPlex™	5,000–25,000	Standard PMT	50

[1] MAGPIX volume can be changed during the run to optimize bead count.

Note: To assure a good bead count, the probe height must be adjusted to the plate provided in the kit. We recommend using two 5.08 mm spacer disks to adjust the sample probe height for Mylar-bottom plates.

Analyze results

The concentration of the samples can be calculated by plotting the expected concentration of the standards against the NET MFI generated by each standard. For Bio-Plex™ Manager, plot standard

concentrations against FI-Bkgd. A 4PL or 5PL algorithm is recommended for the best curve fit. Analyze the assayed samples according to the operation manual for the Luminex™ or Bio-Plex™ instrument.

We offer a free and robust analysis software package for data analysis. To analyze the data, follow the instructions below or contact our technical support.

1. Export the run data in .csv format and navigate to the ProcartaPlex™ Analysis App on Thermo Fisher Connect: https://apps.thermofisher.com/apps/procartaplex

   Note: Before exporting .csv raw data from Bio-Plex™ Manager, please make sure to set 'Analytes Labels' under 'Document Export Properties' to 'Name (Region)'. The .csv raw data exported as Report Type 'xPONENT' from INTELLIFLEX™ instruments are supported.

2. Upload the .csv files to the ProcartaPlex™ Analysis App to analyze the run data. The intuitive software features 4PL/5PL curve fit optimization, group-wise statistical and heat map analysis. Users can export detailed reports including images for presentations and publications.

   IMPORTANT! For ProcartaPlex™ getting started guides, technical literature, protocol support tools, and common troubleshooting questions visit thermofisher.com/procartaplexsupport

   For more complete troubleshooting questions and answers, visit our FAQ database at thermofisher.com/procartaplexfaqs

A Recommended plate layout

Standards		Samples									
1	1	1	1	9	9	17	17	25	25	33	33
2	2	2	2	10	10	18	18	26	26	34	34
3	3	3	3	11	11	19	19	27	27	35	35
4	4	4	4	12	12	20	20	28	28	36	36
5	5	5	5	13	13	21	21	29	29	37	37
6	6	6	6	14	14	22	22	30	30	38	38
7	7	7	7	15	15	23	23	31	31	39	39
Bkgd[1]	Bkgd	8	8	16	16	24	24	32	32	40	40

[1] Background

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

B

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  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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