

ProcartaPlex™ Human Apoptosis Panel 6-Plex

USER GUIDE

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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://www.thermofisher.com/symbols-definition).

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Revision	Date	Description
A.0 (30)	25 February 2021	new manual

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ProcartaPlex™ Human Apoptosis Panel 6-Plex



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](https://www.thermofisher.com/support).

Introduction

The ProcartaPlex™ Human Apoptosis Panel 6-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.

Analytes		
Bcl-2	Caspase-3 (active)	Cleaved PARP
Cytochrome C	GAPDH	p53

For detailed product information, visit [thermofisher.com/procartaplex](https://www.thermofisher.com/procartaplex)

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 12 (lyophilized)	2 each
Caspase 3 Standard (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
Cell Lysate Assay Diluent (1X)	1 x 10 mL
8-Tube Strip	2 each
Flat Bottom 96-well Plate, black	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.



CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Required materials not supplied

- xMAP™ instrument
- Hand-Held Magnetic Plate Washer (Cat. No. EPX-55555-000)
- Deionized water
- Vortex mixer (e.g., Cat. No. 88882010)
- Microcentrifuge
- Adjustable single and multichannel pipettes with disposable tips and low volume reservoirs (e.g., Cat. No. 95128093)

- Beakers, flasks, and cylinders necessary 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 (e.g., Cat. No. 88882006)

Note: The use of rockers or large orbit shakers may cause adverse results.

Precautions and technical hints

1. Thoroughly read this User Guide and Certificate of Analysis prior to using the kit.
2. All chemicals should be considered potentially hazardous.
3. To avoid cross-contamination, do not invert the assay plate during the assay or allow contents from one well to mix with another well.
4. Use a multichannel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.
5. Ensure that the xMAP™ instrument has been properly calibrated and set up prior to preparing and running the assay.

Workflow

Assay protocol

Prepare antigen standard

Add capture beads

1. Vortex capture beads for 30 sec. Add 50 μ L of the capture beads to each well.
2. Remove liquid.

Note: Wash the plate after adding the beads.

Add samples and standards

1. Add the following according to sample type
 - For cell lysate samples:** Add 25 μ L of Assay Diluent, then add 25 μ L of standards or prediluted samples. For background wells, add 50 μ L of 1X Assay Diluent.
2. Seal the plate and incubate with shaking at room temp for 2 hr.
3. Wash plate twice.

Prepare and add detection antibody

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

Add Streptavidin-PE

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

Resuspend beads

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

Acquire data on xMAP™ system

Sample preparation

This assay is suitable for cell lysates only. It is not validated for serum and plasma. For preparing cell lysates, we recommend that you use Invitrogen™ Cell Extraction Buffer (Cat. No. FNN0011) supplemented with 0.5M EDTA and Thermo Scientific™ Halt™ Protease Inhibitor Cocktail (100X) (Cat. No. 78430).

Cell lysate sample preparation

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

1. Stimulate cells as desired, then collect the cells by low-speed centrifugation (400 × *g* for 10 minutes at 4°C).
2. Wash the cells twice in ice-cold PBS, collecting the cells between washes by low-speed centrifugation (400 × *g* for 10 minutes at 4°C).
3. Add 100 µL of Cell Extraction Buffer directly to the dry pellet. The volume of Cell Extraction Buffer depends on the cell number, the level of expression of the target protein, and the level of phosphorylation.
4. Incubate on ice for 30 minutes vortexing every 10 minutes.
5. Transfer the lysate to a microfuge tube, then centrifuge at 13,000 rpm for 10 minutes at 4°C.
6. Aliquot the cleared lysate into clean microfuge tubes. Then determine the total protein concentration.

IMPORTANT!

- Proceed to analysis immediately after collection or freeze and store the cell lysates at –80°C.
 - Avoid multiple freeze-thaw cycles of the frozen cell lysates. Thaw completely, mix well, then clarify by centrifugation at 18,000 × *g* for 5 minutes before analysis to prevent clogging.
 - Cell fractions and whole cell lysates must be diluted at least 10-fold in Assay Diluent before analysis.
-

Dilution of cell lysate samples

Dilute the cell lysate samples 1:10 in Cell Lysate Assay Diluent (1X).

Preparation of reagents

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

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 ddH₂O. 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 Mixes

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. For experiments measuring cell lysate samples, use Cell Lysate Assay Diluent as the diluent to reconstitute and dilute the standard.

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

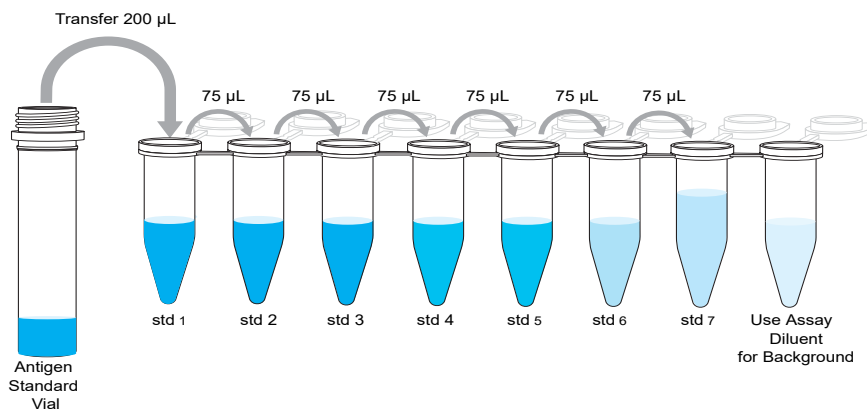
1. Centrifuge both standard mix stock vials at 2,000 x *g* for 10 seconds.
2. Add 125 µL of diluent to each stock vial.
3. Vortex the vials at high speed for 30 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vials.
4. Incubate on ice for 10 minutes to ensure complete reconstitution.
5. Combine the contents of each vial into one of the vials.
6. 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.

Prepare 3-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 75 µL from Std1 tube into Std2 tube.
5. Mix by pipetting up and down 10 times.
6. Transfer 75 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.
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.



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 μ L 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 μ L 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. **Cell lysates:** Add 25 μ L of 1X Assay Diluent to each well followed by 25 μ L of prepared standards or prediluted samples as defined on the plate layout. Add an additional 25 μ L of 1X Assay Diluent to the wells designated as backgrounds.
 - 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.

Note: For those wishing to perform the assay over two days, the 96-well plate can be incubated overnight. Shake the 96-well plate for 30 minutes at room temperature at 600 rpm, then transfer the plate to 4°C and store on a level surface. After overnight incubation, shake the plate for an additional 30 minutes at room temperature at 600 rpm.

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 μ L 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 Detection Antibody Mix to the plate.
 - a. Using a multichannel pipette, add 25 μ L of the detection antibody solution (1X) 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 μ L 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 a xMAP™ instrument.
 - a. Add 120 μ L 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 a xMAP™ instrument.

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™	4,000–13,000	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.

Note: The samples have been diluted 1:10, which must be accounted for in the software analysis.

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



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												

