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ProcartaPlex™ Multiplex Immunoassay

USER GUIDE

Using Magnetic Beads for Serum, Plasma, and Cell Culture Supernatant Samples

Instructions for Human High Sensitivity Assays

Publication Number MAN0019074

Revision A.0 (31)

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ThermoFisher SCIENTIFIC

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Revision history: Pub. No. MAN0019074

Revision	Date	Description
A.0 (31)	07 April 2020	Baseline for this revision history.

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Contents

ProcartaPlex™ Multiplex Immunoassay

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 use

This user manual is for a ProcartaPlex™ Immunoassay Kit to perform quantitative, multiplexed protein measurements from serum, plasma, and cell culture supernatant samples using magnetic beads technology from Luminex™. Other biological samples might be suitable for use in the assay.

For the most current version of user documentation, visit our website.

How it works

ProcartaPlex™ Immunoassays incorporate magnetic microsphere technology licensed from the Luminex™ Corporation to enable the simultaneous detection and quantitation of multiple protein targets in diverse matrices. The platform allows the simultaneous detection from a single sample of up to 80 protein targets on the Luminex™ 200™ and FLEXMAP 3D™ platforms and 50 protein targets on the MAGPIX™ platform.

Materials provided and storage conditions

ProcartaPlex™ Immunoassay Kits contain the components listed below. Refer to the Certificate of Analysis for quantities and details of components supplied. Store kit at 2–8°C. Expiration date is stated on the kit. Do not use after expiration date.

Components supplied	Pre-mixed panels	Simplex kit	Basic kit	Mix&Match panels
Antigen Standards, premixed	✓	✓		✓
Detection Antibody (50X)[1]	✓	✓		
Detection Antibody, premixed (1X)[1]				✓
Antibody Coupled Magnetic Beads, Simplexes (50X)[1]		✓		
Antibody Coupled Magnetic Beads, premixed (1X)[1]	✓			✓
Streptavidin-PE (SA-PE) (1X)[1]	✓		✓	✓
Amplification Reagent 1	✓		✓	✓
Amplification Reagent 2	✓		✓	✓
Wash Buffer Concentrate (10X)[1]	✓		✓	✓
Detection Antibody Diluent[1]	✓		✓	
Universal Assay Buffer (1X)[1]	✓		✓	✓
Reading Buffer[1]	✓		✓	✓
8-Tube Strip	✓		✓	✓
96-Well Flat Bottom Plate	✓		✓	✓
Black Microplate Lid	✓		✓	✓
Plate Seals	✓		✓	✓

[1] Contains sodium azide. See WARNING.

WARNING! All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice. This kit contains small quantities of sodium azide. Sodium azide is highly toxic and reactive in the pure form. At this product's concentration, though not classified as hazardous, buildup of sodium azide may react with lead and copper plumbing to form highly reactive explosive metal azide. Dispose of the product in accordance with all state and local regulations.

Precautions and technical hints

- Thoroughly read this user manual and Certificate of Analysis that is included with the assay kit. The product insert may contain specific instructions for proper use of your kit.
- For Luminex™ 200™ and FLEXMAP 3D™ instruments initiate the startup protocol to warm up the lasers for at least 30 minutes. Ensure that the Luminex™ machine is calibrated according to the manufacturer's instructions. MAGPIX™ instrument doesn't require additional warm up.
- When working with samples and standards, change the pipette tips after every transfer and avoid creating bubbles when pipetting.
- During the incubation steps, cover the 96-well Flat Bottom Plate with the Black Microplate Lid provided in the kit to minimize exposure of the beads to light.
- Be careful not to invert the 96-well Flat Bottom Plate during the assay or allow contents from one well to mix with another well.
- Use a multi-channel pipette and reagent reservoirs whenever possible to achieve optimal assay precision.
- Store the reconstituted standards on ice before adding to the 96-well Flat Bottom Plate.

Required equipment and materials not supplied

- MAGPIX™, Luminex™ 200™, FLEXMAP 3D™, or Luminex™-based instrument.
- Glass-distilled or deionized water.
- Adjustable single and multichannel pipettes with disposable tips.
- Multichannel pipette reservoir.
- Beakers, flasks, and cylinders necessary for preparation of reagents.
- Hand-Held Magnetic Plate Washer, vortex mixer, and microtiter plate shaker.

IMPORTANT! Use only single-use trays for all reagents and buffers.

Sample preparation

- For frozen samples, thaw samples on ice and mix well by vortexing followed by centrifugation at 10,000 × *g* for 5–10 minutes to remove particulates. Avoid multiple freeze/thaw cycles.
- If samples are high in lipid content, centrifuge at 10,000 × *g* for 10 minutes at 2-8℃ and transfer contents to a new tube.

Plasma sample preparation

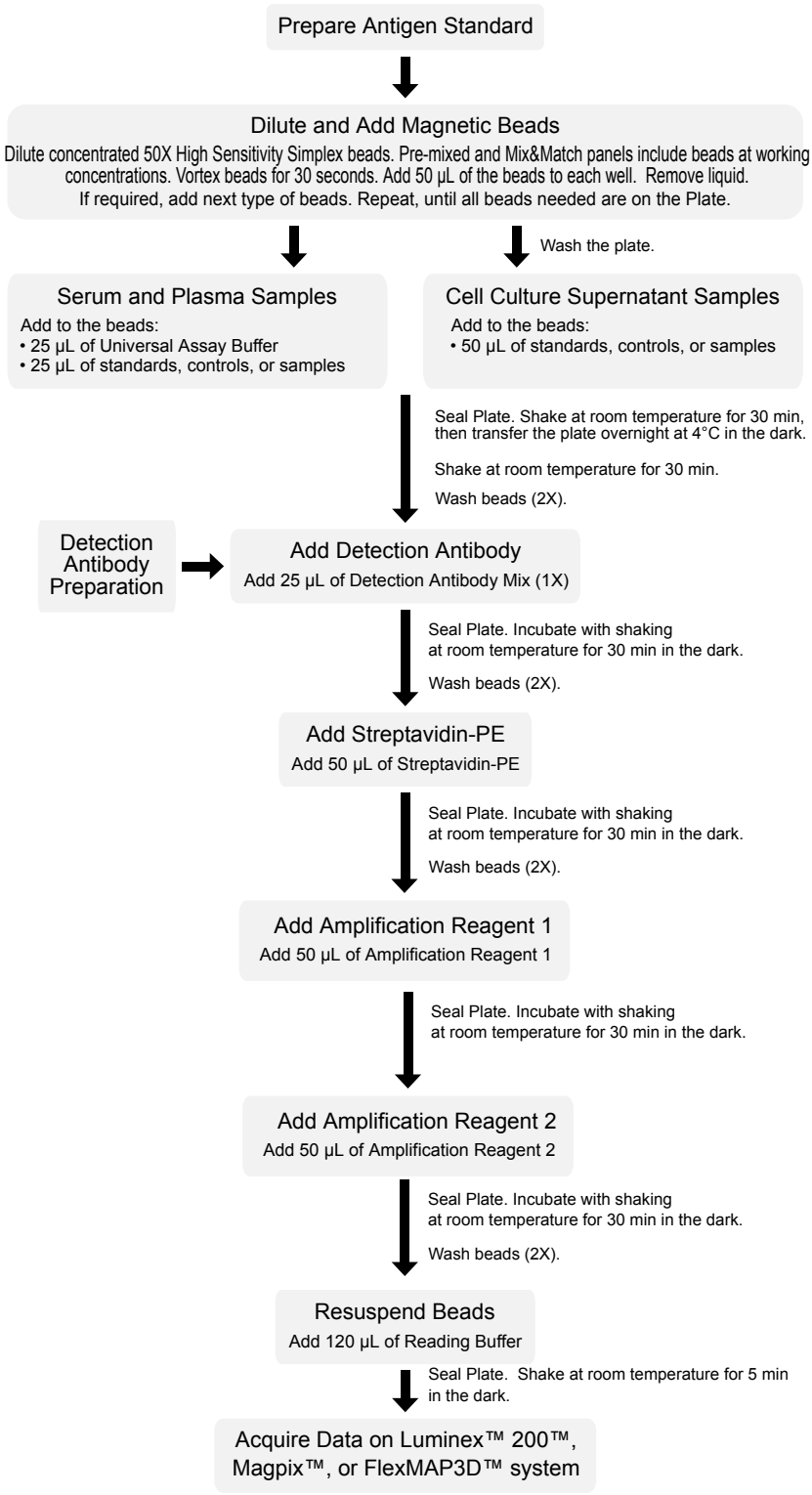
1. Collect samples in sodium citrate or EDTA tubes. When using heparin as an anticoagulant, no more than 10 IU of heparin per mL of blood collected should be used because an excess of heparin may give falsely high values of some of the analytes.
2. Centrifuge samples at 1,000 × *g* at 4°C for 10 minutes within 30 minutes of collection.
3. Collect the plasma fraction. Use immediately or aliquot and store at –80°C.

Serum sample preparation

Spin down serum samples at 1,000 × *g* for 10 minutes at 20–25°C before running the assay.

1. Allow blood to clot for 20–30 minutes at 20–25°C.
2. Centrifuge at 1,000 × *g* for 10 minutes at 20–25°C.
3. Collect the serum fraction. (Alternatively, use any standard serum separator tube following the manufacturer's instructions.)
4. Use immediately or aliquot and store at –80°C.

Assay protocol overview



Preparation of reagents

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. Wash Buffer (1X) can be stored at 2–8°C for up to 6 months.

Note: Wash Buffer Concentrate volume might not be sufficient if using automated plate washer. For bulk orders use Cat. No. EPX-66666-001.

Prepare 1X High Sensitivity Simplex Beads

High Sensitivity Simplex kits are provided with concentrated 50X beads, and dilution of concentrated High Sensitivity Simplex beads is required.

1. Vortex each High Sensitivity Simplex Bead vial (50X) for 30 second, then add 100 µL of each High Sensitivity Simplex Bead vial (50X) to a mixing bottle if using a whole plate (otherwise adjust the volume accordingly).

2. Fill up with Wash Buffer (1X) to a final volume of 5 mL.

   To combine 2 or more different bead vials follow the table below (using a whole plate):

Number of different Simplex Bead Vials to be mixed	Total volume of mixed bead solution	Volume of Wash Buffer (1X) to add
1	100 µL	4900 µL
2	200 µL	4800 µL
3	300 µL	4700 µL
4	400 µL	4600 µL
5	500 µL	4500 µL
6	600 µL	4400 µL

Prepare 1X Detection Antibody Mixture

Detection antibody is provided at a 50X concentration in the High Sensitivity Simplex and Pre-mixed panels (50X) and requires dilution prior to use.

IMPORTANT! High Sensitivity Mix&Match Panels contain 1X detection antibody and does not require any further dilution.

1. Add 60 µL of each Detection Antibody concentrate to a mixing bottle.
2. Fill up with Detection Antibody Diluent to a final volume of 3 mL if using the whole plate.

   If you are not using the whole plate, adjust the volume accordingly using the following table:

Number of vials of Detection Antibody	Total volume Detection Antibody	Volume of Diluent to add
1	60 µL	2940 µL
2	120 µL	2880 µL
3	180 µL	2820 µL
4	240 µL	2760 µL
5	300 µL	2700 µL
6	360 µL	2640 µL

Prepare antigen standard

Carefully read the Certificate of Analysis for lot-specific information on the kit components. The majority of kits is supplied with lyophilized multistandards containing a mix of multiple standard proteins. Some kits contain multiple sets of standards, each with a unique lot number, that require pooling prior to use. Each kit is shipped with 2 identical vials of each premixed antigen standard set from the same lot to permit the user to run the assay twice if running a partial plate. When preparing Antigen Standards, the final volume after reconstitution and pooling should be 250 µL. For instructions for combining more than 5 antigen standard sets contact our technical support.

Reconstitute and pool standards

1. Centrifuge each different antigen standard set vial(s) at 2,000 x *g* for 10 seconds.
2. Add 50 µL of sample type specific buffer into each standard vial. If you want to measure serum or plasma samples, use Universal Assay Buffer (1X) to reconstitute the standard, if you want to analyze cell culture supernatant samples, use the cell culture medium that was used to culture the cells to dissolve the standard.
3. Gently vortex the vial(s) for 10 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents at the bottom of the vial(s).
4. Incubate on ice for 10 min to ensure complete reconstitution.

5. Pool entire contents of each vial into one of the vials and fill up with sample type specific buffer to a total volume of 250 µL.

6. Gently vortex the vial for 10 seconds and centrifuge at 2,000 x *g* for 10 seconds to collect contents on the bottom of the vial.

# of Standard sets	Reconstitution volume per vial	Pooled volume	Buffer to add	Total volume
1	50 µL	50 µL	200 µL	250 µL
2	50 µL	100 µL	150 µL	250 µL
3	50 µL	150 µL	100 µL	250 µL
4	50 µL	200 µL	50 µL	250 µL
5	50 µL	250 µL	0 µL	250 µL

7. Predilute the reconstituted antigen standard 1:10 (25 µL of reconstituted antigen standard with 225 µL diluent). For serum or plasma samples, use Universal Assay Buffer and for cell culture supernatant samples use the cell culture media that was used to culture the cells.

Prepare 4-fold serial dilution

1. Prepare a 4-fold serial dilution of the reconstituted standard(s) using the 8-tube strip provided. Label tubes Std1, Std2, Std3, Std4, Std5, Std6, and Std7.

2. Add 200 µL of the reconstituted and prediluted antigen standard into the first tube of the strip and label as Standard 1 (Std1).

3. Add 150 µL of sample type specific standard buffer into Std tubes 2–7. Use Universal Assay Buffer for serum or plasma samples and cell culture media for culture supernatant samples.

4. Transfer 50 µL of the reconstituted and prediluted antigen standard from Tube 1 into Tube 2.

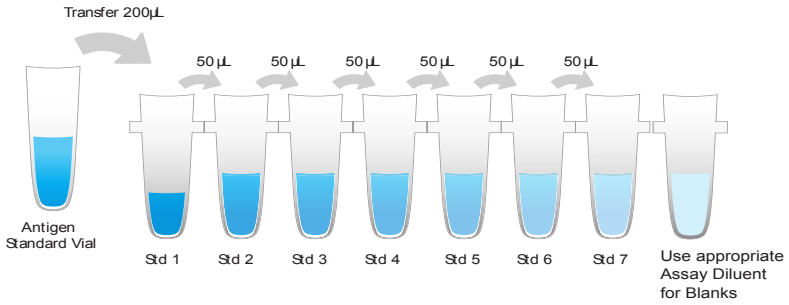
5. Mix by pipetting up and down for a total of 10 times.

6. Transfer 50 µL of the mixed standards from Tube 2 into Tube 3.

7. Mix by pipetting up and down for a total of 10 times.

8. Repeat steps 4–7 for Std tubes 4–7.

9. Add 200 µL of Universal Assay Buffer or cell culture medium into tube 8, which serves as a blank. Keep on ice until ready to use.



Assay protocol

IMPORTANT! Use only single-use trays for all reagents and buffers.

1. Define the plate map.

   Mark the standard, sample, and blank wells using the plate map at the end of this manual.

2. Add magnetic beads to the plate.

   Note: High Sensitivity Pre-mixed and Mix&Match Panels contain only one bead vial at working concentration 1X.

   a. Vortex the Magnetic Bead vial for 30 seconds.

   b. Add 50 µL of the Magnetic Bead solution to each well of the plate. Use a multichannel pipette for this step as well as for the steps below.

3. Wash magnetic beads.

   a. Securely insert the 96-well Flat Bottom Plate into the Hand-Held Magnetic Plate Washer, ensure that the plate is held in place by the tabs, and wait 2 minutes to allow the beads to accumulate on the bottom of each well.

   b. Remove the liquid in the wells by quickly inverting the Hand-Held Magnetic Plate Washer and 96-well Flat Bottom Plate assembly over a sink or waste container. Do not remove the 96-well Flat Bottom Plate from the Hand-Held Magnetic Plate Washer. Blot the inverted assembly onto several layers of paper towels or absorbent surface to remove any residual solution.

   c. Add 150 µL of Wash Buffer (1X) into each well and wait 30 seconds to allow the beads to accumulate on the bottom of each well.

   d. Remove the Wash Buffer in the wells by quickly inverting the Hand-Held Magnetic Plate Washer and 96-well Flat Bottom Plate assembly over a sink or waste container. Do not remove the 96-well Flat Bottom Plate from the Hand-Held Magnetic Plate Washer. Blot the inverted assembly onto several layers of paper towels or absorbent surface to remove any residual solution.

e. Remove the 96-well Flat Bottom Plate from the Hand Held Magnetic Plate Washer and proceed to the next step.

4. Add sample type-specific buffer, samples, standards, and blanks, and then incubate.
   a. For serum and plasma: Add 25 µL of Universal Assay Buffer (1X) to each well followed by 25 µL of prepared standards or samples into dedicated wells. For cell culture supernatants: Add 50 µL prepared standards or samples into dedicated wells.

   b. For wells designated as blanks: Add an additional 25 µL of Universal Assay Buffer for serum or plasma samples. For cell culture supernatant samples, add 50 µL of cell culture medium.

   c. Incubate the 96-well plate overnight. Shake the 96-well plate for 30 minutes at room temperature at 500 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 500 rpm.

5. Wash the 96-well plate twice following step 3.

6. Add Detection Antibody Mixture and incubate.
   a. Add 25 µL of Detection Antibody Mixture (1X) to each well.

   b. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, and incubate 30 minutes on a plate shaker at room temperature at 500 rpm.

7. Wash the 96-well plate twice following step 3.

8. Add SAPE and incubate.
   a. Add 50 µL of SAPE solution to each well.

   b. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, and incubate 30 minutes on a plate shaker at room temperature at 500 rpm.

9. Wash the 96-well plate twice following step 3.

10. Add Amplification Reagent 1.
    a. Add 50 µL of Amplification Reagent 1 into each well.

    b. Seal the plate with a new Plate Seal, remove the plate from Hand-Held Magnetic Plate Washer, cover the plate with the Black Microplate Lid, and incubate 30 minutes on a plate shaker at room temperature at 500 rpm.

    After incubation immediately proceed to next step. DO NOT WASH.

11. Add Amplification Reagent 2.
    a. Add 50 µL of Amplification Reagent 2 into each well.

b. Seal the plate with a new Plate Seal, cover plate with the Black Microplate Lid, and incubate 30 minutes on a plate shaker at room temperature at 500 rpm.

12. Wash the 96-well plate twice following step 3.

13. Prepare the 96-well plate for analysis on a Luminex™ instrument.
    a. Add 120 µL of Reading Buffer into each well.

    b. Seal the plate with a new Plate Seal, cover the plate with the Black Microplate Lid, and incubate 5 minutes on a plate shaker at room temperature at 500 rpm.

    c. Remove Plate Seal and run the plate on a Luminex™ instrument.

Setup of the instruments

Instrument	Sample size	DD gate	Timeout	Bead event/bead region
Luminex™ 200™ FLEXMAP 3D™	50 µL	5,000–25,000	60 seconds	50–100
MAGPIX™	50 µL	N/A	N/A	50–100

Prior to running the assay, ensure that the probe height has been calibrated with 96-well Flat Bottom Plate supplied with the kit. Failure to adjust the probe height can cause damage to the instrument or low bead count. The Luminex™ system allows for calibration of low and high RP1 target values. We recommend RP1 low target value settings for ProcartaPlex™ immunoassays. When entering the information into the Luminex™ Acquisition Software, refer to the Certificate of Analysis provided with the kit for bead region and S1 values for each analyte of the current lot.

Note: If there is a malfunction of the Luminex™ instrument or software during the run, the 96-well Flat Bottom Plate can be re-read. Remove the 96-well Flat Bottom Plate from the instrument, insert the 96-well Flat Bottom Plate into the Hand-Held Magnetic Plate Washer, wait 2 minutes, then remove the buffer in the wells by quickly inverting the 96-well Flat Bottom Plate over a sink or waste container. Blot the assembly onto several layers of paper towels to remove any residual solution. Resuspend the beads in 120 µL of Reading Buffer, remove from the Hand-Held Magnetic Plate Washer, seal the 96-well Flat Bottom Plate with a new Plate Seal and Lid and shake at 500 rpm for 5 minutes at room temperature. The assayed samples may take longer to read since there will be less beads in the well.

Analyzing results

The concentration of the samples can be calculated by plotting the expected concentration of the standards against the MFI generated by each standard. A 4PL or 5PL algorithm is recommended for the best curve fit. Analyze the assayed samples according to the operation manual for the Luminex™ instrument (e.g., MAGPIX™, Luminex™ 200™, FLEXMAP 3D™). We offer a free and robust analysis software package for data analysis. For download information visit our website or contact our technical support.

Troubleshooting

Observation	Probable cause	Recommend solution
Low Flow Rate	Samples/beads are stuck in flow cell	Remove the 96-well Plate and perform a wash and rinse cycle.
High CVs	Samples and antigen standards not stored on ice	Prepare the samples and standards on ice before setting up the assay.
	Contamination from reusing the Plate Seal	Use a new Plate Seal for each incubation step.
	Incomplete washing	After adding the standards and samples, it is very important that any excess standards are removed during the wash step.
	Contamination from contents from adjacent wells	Avoid splashing the Wash Buffer during wash steps into adjacent wells.
	Poor pipetting techniques	Use a multichannel pipettor and careful pipette techniques. Avoid touching pipette tips to sides of the wells when adding Wash Buffer.
Limited dynamic range for BioPlex software users	Instrument calibrated at high PMT settings	Calibrate the instrument using the CAL2 Low RP1 target value.
Low bead count	Volume of bead solution is too low	Add 120 µL Reading Buffer into each well and shake at 500 rpm for 5 minutes at room temperature to resuspend beads prior to reading on the Luminex™ instrument.
	High bead aggregation	Vortex the bead suspension well before using in the assay and ensure that the beads are properly mixed during the incubation steps.
	Dyes contained in the beads are photo-bleached from overexposure to light	Store bead solution and the 96-well plate in the dark.

Observation	Probable cause	Recommend solution
Low bead count	Samples causing the instrument to clog	Remove the 96-well Flat Bottom Plate and perform a wash and rinse to the instrument. Rerun the assay with further dilution of samples
	Probe height is incorrect	Refer to the Luminex™ Manual for proper adjustment of the needle height.
	Instrument needle is partially clogged	Replace or clean needle according to the manufacturer's recommendations.
	Beads stuck to the bottom of the plate	Confirm that the plate shaker is set to 500 rpm and shaking for at least 5 minutes before reading.
	Air bubble in the sample loop	Refer to the Luminex™ manual for proper removal of the air bubble.
Low signal or sensitivity	Standards not reconstituted and diluted correctly	Prepare fresh antigen standards following the instructions in "Prepare antigen standard" on page 11
Poor recovery	Did not use appropriate cell culture media to prepare the standards	Use the same cell culture media that is used to culture the cells.
	Samples and antigen standards were not stored on ice	Prepare the samples and standards on ice before setting up the assay.

Recommended and blank 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
Blank	Blank	8	8	16	16	24	24	32	32	40	40

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

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  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.