Bio-Plex Pro™ RBM Apoptosis Assays
Instruction Manual

For technical support, call your local Bio-Rad office, or in the U.S., call 1-800-424-6723.
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Introduction

Apoptosis refers to a genetically controlled process by which cells die following a programmed physiological state or pathological condition. The balance between pro- and anti-apoptotic processes is delicate, and dysregulation is observed in a wide range of pathological conditions (Rutledge 2002). Excessive apoptosis is implicated in neurodegenerative and autoimmune diseases, myocardial infarction, stroke, and viral infection. In contrast, insufficient apoptosis is linked to almost every known human cancer.

Cell death activation is thought to be regulated by the Bcl-2 family of proteins, which consists of both pro-apoptotic and anti-apoptotic members. At least 17 human proteins with Bcl-2 homology have already been identified. Although much has been studied about Bcl-2 family–mediated apoptosis, there is clearly more to discover, specifically the mechanism by which heterodimerization regulates both pro- and anti-apoptotic proteins (Rutledge 2002).

Apoptosis is induced by at least two distinct signaling pathways. The extrinsic pathway is triggered by cell signaling through death receptors such as Fas, followed by downstream activation of caspase-8 and caspase-3. The intrinsic pathway is triggered by cytotoxic stress, which leads to translocation of pro-apoptotic Bcl-2 proteins such as Bax and Bak to the mitochondrial membrane. This causes release of cytochrome C into the cytosol, where it promotes apoptosome formation and final activation of caspase-3.

Bio-Plex Pro™ RBM Apoptosis Assays
The Bio-Plex Pro RBM apoptosis assays enable researchers to quantify multiple intracellular proteins involved in the commitment, onset, and induction of apoptosis by the intrinsic pathway. Compatible sample types include cell lysates and tissue homogenates.

The use of magnetic (MagPlex) beads allows researchers to automate wash steps on a Bio-Plex Pro (or similar) wash station. Magnetic separation offers greater convenience, productivity, and reproducibility compared to vacuum filtration.

References
Principle

Technology
The Bio-Plex® multiplex system is built upon the three core elements of xMAP technology:

- Fluorescently dyed magnetic microspheres (also called beads), each with a distinct color code or spectral address to permit discrimination of individual tests within a multiplex suspension. This allows simultaneous detection of up to 500 different molecules in a single well of a 96-well microplate on the Bio-Plex® 3D system, up to 100 different molecules on the Bio-Plex® 200 system, and up to 50 different molecules on the Bio-Plex® MAGPIX™ system.

- A dedicated plate reader. The Bio-Plex 200 and Bio-Plex 3D systems are flow cytometry–based instruments with two lasers and associated optics to measure the different molecules bound to the surface of the beads. In the Bio-Plex MAGPIX system, the entire sample load volume is injected into a chamber where the beads are imaged using LED and CCD technology.

- A high-speed digital signal processor that efficiently manages the fluorescence data.

Assay Format
The Bio-Plex Pro™ RBM apoptosis assays are essentially immunoassays formatted on magnetic beads. The assay principle is similar to that of a sandwich ELISA (Figure 1). Capture antibodies directed against the desired biomarker are covalently coupled to the beads. Coupled beads react with the sample containing the biomarker of interest. After a series of washes to remove unbound protein, a biotinylated detection antibody is added to create a sandwich complex. The final detection complex is formed with the addition of streptavidin-phycoerythrin (SA-PE) conjugate. Phycoerythrin serves as a fluorescent indicator, or reporter.
**Data Acquisition and Analysis**

Data from the reactions are acquired using a Bio-Plex system or similar Luminex-based reader. When a multiplex assay suspension is drawn into the Bio-Plex 200 reader, for example, a red (635 nm) laser illuminates the fluorescent dyes within each bead to provide bead classification and thus assay identification. At the same time, a green (532 nm) laser excites PE to generate a reporter signal, which is detected by a photomultiplier tube (PMT). A high-speed digital processor manages data output and Bio-Plex Manager™ software presents data as median fluorescence intensity (MFI) as well as concentration. The concentration of analyte bound to each bead is proportional to the MFI of reporter signal.
Kit Contents and Storage

The Bio-Plex Pro™ RBM apoptosis assays are available in a convenient kit format that includes assay, reagent, and diluent components in a single box (Table 1). All other recommended materials are listed in Table 2.

Table 1. Contents of 1 x 96-well kits.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Volume</th>
<th>Volume after Reconstitution or Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture beads (1x)</td>
<td>1 tube</td>
<td>1.4 ml</td>
<td></td>
</tr>
<tr>
<td>Detection antibodies</td>
<td>1 vial</td>
<td>Lyophilized</td>
<td>4.8 ml</td>
</tr>
<tr>
<td>Standards mix</td>
<td>1 vial</td>
<td>Lyophilized</td>
<td>150 µl</td>
</tr>
<tr>
<td>Control 1 (high)</td>
<td>1 vial</td>
<td>Lyophilized</td>
<td>100 µl</td>
</tr>
<tr>
<td>Control 2 (low)</td>
<td>1 vial</td>
<td>Lyophilized</td>
<td>100 µl</td>
</tr>
<tr>
<td>Blocking buffer</td>
<td>1 vial</td>
<td>Lyophilized</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Standard diluent</td>
<td>1 vial</td>
<td>Lyophilized</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Assay buffer (10x)</td>
<td>1 bottle</td>
<td>60 ml</td>
<td>600 ml</td>
</tr>
<tr>
<td>Lysate dilution buffer (LDB)*</td>
<td>1 bottle</td>
<td>100 ml</td>
<td></td>
</tr>
<tr>
<td>Cytosolic extraction buffer (CEB)*</td>
<td>1 bottle</td>
<td>60 ml</td>
<td></td>
</tr>
<tr>
<td>Streptavidin-PE (10x)</td>
<td>1 tube</td>
<td>250 µl</td>
<td>2.5 ml</td>
</tr>
<tr>
<td>Assay plate (96-well flat bottom)</td>
<td>1 plate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plate seals</td>
<td>1 pack of 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assay quick guide</td>
<td>1 sheet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Product data sheet</td>
<td>1 sheet</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Protease inhibitors not included.

Storage and Stability

Kit contents should be stored at 2–8°C and never frozen. Coupled magnetic capture beads and streptavidin-PE should be stored in the dark. All components are guaranteed for a minimum of six months from the date of purchase when stored as specified.

4
Table 2. Recommended materials.

<table>
<thead>
<tr>
<th>Item</th>
<th>Ordering Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Plex® 200 system or Luminex system with HTF</td>
<td>Bio-Rad catalog #171-000205</td>
</tr>
<tr>
<td>Bio-Plex validation kit</td>
<td>Bio-Rad catalog #171-203001</td>
</tr>
<tr>
<td>Note: Run the validation kit monthly to ensure optimal performance of fluidics and optics systems</td>
<td></td>
</tr>
<tr>
<td>Bio-Plex calibration kit</td>
<td>Bio-Rad catalog #171-203060</td>
</tr>
<tr>
<td>Note: Run the calibration kit daily to standardize fluorescence signal</td>
<td></td>
</tr>
<tr>
<td>Bio-Plex Pro wash station</td>
<td>Bio-Rad catalog #300-34376</td>
</tr>
<tr>
<td>For use with magnetic bead–based assays only</td>
<td></td>
</tr>
<tr>
<td>Bio-Plex Pro II wash station</td>
<td>Bio-Rad catalog #300-34377</td>
</tr>
<tr>
<td>For use with both polystyrene (nonmagnetic) and magnetic bead–based assays</td>
<td></td>
</tr>
<tr>
<td>Bio-Plex handheld magnetic washer</td>
<td>Bio-Rad catalog #170-20100</td>
</tr>
<tr>
<td>For use with magnetic bead–based assays only</td>
<td></td>
</tr>
<tr>
<td>Bio-Plex Pro flat bottom plates (40 x 96-well)</td>
<td>Bio-Rad catalog #171-025001</td>
</tr>
<tr>
<td>For magnetic separation on the Bio-Plex Pro wash station</td>
<td></td>
</tr>
<tr>
<td>Titertube® micro test tubes</td>
<td>Bio-Rad catalog #223-9390</td>
</tr>
<tr>
<td>For preparing replicate standards, samples, and controls prior to loading the plate</td>
<td></td>
</tr>
<tr>
<td>Microtiter plate shaker</td>
<td>IKA catalog #320-8000</td>
</tr>
<tr>
<td>IKA MTS 2/4 shaker for 2 or 4 microplates</td>
<td></td>
</tr>
<tr>
<td>or</td>
<td>VWR catalog #57019-600</td>
</tr>
<tr>
<td>Barnstead/Lab-Line Model 4625 plate shaker (or equivalent capable of 300–1,100 rpm)</td>
<td></td>
</tr>
<tr>
<td>Aurum™ vacuum manifold</td>
<td>Bio-Rad catalog #732-6470</td>
</tr>
<tr>
<td>For vacuum filtration</td>
<td></td>
</tr>
<tr>
<td>BR-2000 vortexer</td>
<td>Bio-Rad catalog #166-0610</td>
</tr>
<tr>
<td>Reagent reservoirs, 25 ml</td>
<td>VistaLab catalog #3054-1002</td>
</tr>
<tr>
<td>For capture beads and detection antibodies</td>
<td>or</td>
</tr>
<tr>
<td>VistaLab catalog #3054-1004</td>
<td></td>
</tr>
<tr>
<td>Reagent reservoir, 50 ml (for reagents and buffers)</td>
<td>VistaLab catalog #3054-1004</td>
</tr>
<tr>
<td>Pall Life Science Acrodisc: 25 mm PF syringe filter</td>
<td>Pall Life Sciences catalog #4187</td>
</tr>
<tr>
<td>(0.8/0.2 µm Supor membrane)</td>
<td></td>
</tr>
<tr>
<td>Filter plate, 1 x 96 with clear plastic lid and tray</td>
<td>Bio-Rad catalog #171-304502</td>
</tr>
</tbody>
</table>

Other: 15 ml polypropylene tubes for reagent dilutions, calibrated pipets, pipet tips, sterile distilled water, aluminum foil, absorbent paper towels, 1.5 or 2 ml microcentrifuge tubes, and standard flat bottom microplate (for calibrating vacuum manifold).
Reconstitute lyophilized reagents, dilute assay buffer to 1x, prepare standards and samples

Add 10 µl blocking buffer to all wells

Add 30 µl standards, blank, samples, and controls to appropriate wells

Add 10 µl 1x capture beads per well. Incubate at 850 ± 50 rpm for 1 hr at RT

Wash 3x with 100 µl assay buffer (1x)

Add 40 µl reconstituted detection antibodies. Incubate at 850 ± 50 rpm for 1 hr at RT

Do not aspirate after incubation

Add 20 µl 1x streptavidin-PE. Incubate at 850 ± 50 rpm for 30 min at RT

Wash 3x with 100 µl assay buffer (1x)

Resuspend beads in 100 µl assay buffer (1x). Shake at 850 ± 50 rpm for 30 sec

Read plate on Bio-Plex system
Important Considerations

Instruments and Software
The assays described in this manual are compatible with all currently available Luminex-based life science research instruments. Assays can be read and analyzed with either Bio-Plex Manager™ software or Luminex xPONENT software.

Assay Procedures
Pay close attention to vortexing, shaking, and incubation times and to Bio-Plex® reader PMT (RP1) setting, as these have been optimized specifically for each assay panel.

Assay Quick Guide
Each assay kit comes complete with a printed Bio-Plex Pro RBM Apoptosis Assays Quick Guide (bulletin #10033632), which can be used to set up and prepare a full 1 x 96-well assay plate. Users can also download a copy at www.bio-rad.com/bio-plex.

Bead Regions and Multiplexing Compatibility
- Bead regions for all analytes are listed in the Read Plate section
- Do not mix analytes between different apoptosis panels, or with other Bio-Plex assay panels or reagents

Detailed Instructions

The following pages provide detailed instructions for each step of the assay procedure, including preparation, running the assay, and reading the plate with Bio-Plex Manager™ and Luminex xPONENT software.
1. Plan Plate Layout

Determine the total number of wells in the experiment using the Plate Layout Template on page 32 or the Plate Formatting tab in Bio-Plex Manager™ software. A suggested plate layout is shown in Figure 2, with all conditions in duplicate.

1. Assign standards to columns 1 and 2, with the highest concentration in row A and the lowest concentration in row H.

2. Assign the blank to wells A3 and A4. The blank should consist of standard diluent alone and be processed in the same manner as sample and standard wells. Bio-Plex Manager™ software automatically subtracts the assay blank (B) MFI value from all other assay wells.

3. User-specific controls, as well as the quality controls supplied in the kits, are assigned to wells in columns 3 and 4.

4. The remainder of the plate is available for samples.

Fig. 2. Suggested plate layout. For detailed instructions on plate formatting in Bio-Plex Manager, see section Read Plate.

Legend

- S: Standards
- B: Blank
- X: Samples
- C: Controls
2. Prepare Instrument

These directions are specific for the Bio-Plex® 100/200 reader. To prepare either a Bio-Plex 3D or Bio-Plex® MAGPIX™ reader, consult their respective user manuals.

Start up and calibrate the Bio-Plex® system with Bio-Plex Manager™ software prior to setting up the assay. The calibration kit should be run daily or before each use of the instrument to standardize the fluorescent signal. For instructions on using other xMAP system software packages, contact Bio-Rad Technical Support.

**Note:** While the instrument is warming up, bring the 10x assay buffer and sample dilution buffer to room temperature. Keep other items on ice until needed. Also, begin to thaw frozen samples.

The validation kit should be run monthly to ensure performance of fluidics and optics systems. Refer to either the software manual or online Help for directions on how to conduct validation.

**Start Up System (Bio-Plex 100, 200, or similar)**

1. Empty the waste bottle and fill the sheath fluid bottle before starting if high throughput fluidics (HTF) are not present. This will prevent fluidic system backup and potential data loss.

2. Turn on the reader, XY platform, and HTF (if included). Allow the system to warm up for 30 min (if not already done).

3. Select Start up and follow the instructions. If the system is idle for 4 hr without acquiring data, the lasers will automatically turn off. To reset the 4-hr countdown, select Warm up and wait for the lasers/optics to reach operational temperature.
Calibrate System

1. Select Calibrate and confirm that the default values for CAL1 and CAL2 are the same as the values printed on the bottle of Bio-Plex calibration beads. Use the Bio-Plex system low RP1 target value.

2. Select OK and follow the software prompts for step-by-step instructions for CAL1 and CAL2 calibration.

Note: In Bio-Plex Manager version 6.1 and higher, startup, warm up, and calibration can be performed together by selecting the “Start up and calibrate” icon.

3. Prepare Wash Method

Bio-Plex Pro™ assays are compatible with both magnetic separation and vacuum filtration methods. However, for best results, we recommend performing the assays in a flat bottom plate with magnetic separation.

Table 3. Summary of compatible wash stations and plate types.

<table>
<thead>
<tr>
<th>Wash Method</th>
<th>Wash Station</th>
<th>Assay Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic separation</td>
<td>Bio-Plex Pro</td>
<td>Flat bottom plate</td>
</tr>
<tr>
<td></td>
<td>Bio-Plex Pro II (use MAG programs)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bio-Plex® handheld magnetic washer</td>
<td></td>
</tr>
<tr>
<td>Vacuum filtration</td>
<td>Bio-Plex Pro II (use VAC programs)</td>
<td>Filter plate</td>
</tr>
<tr>
<td></td>
<td>Vacuum manifold (manual)</td>
<td></td>
</tr>
</tbody>
</table>

Setting up the Bio-Plex Pro or Bio-Plex Pro II Wash Station

The wash station should be primed before use. For more information, refer to the Bio-Plex Pro Wash Stations Quick Guide (bulletin #5826).

1. Install the appropriate plate carrier on the wash station.

2. Use the prime procedure to prime channel 1 with 1x assay buffer.
Setting Up the Bio-Plex Handheld Magnetic Washer

Place an empty flat bottom plate on the magnetic washer by sliding it under the retaining clips. Push the clips inward to secure the plate. Make sure the plate is held securely. If needed, the clips can be adjusted for height and tension. For detailed instructions, refer to the user guide (bulletin #10023087).

Setting up a Vacuum Manifold

Calibrate the vacuum manifold by placing a standard 96-well flat bottom plate on the unit and adjusting the pressure to –1 to –3” Hg. In general, 100 µl liquid should take 3–4 sec to clear the well. For more detailed instructions, refer to bulletin #10005042.

4. Prepare Reagents

1. Reconstitute the following lyophilized reagents in dH₂O before use according to the table below.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>dH₂O Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards mix</td>
<td>150 µl</td>
</tr>
<tr>
<td>Control 1</td>
<td>100 µl</td>
</tr>
<tr>
<td>Control 2</td>
<td>100 µl</td>
</tr>
<tr>
<td>Blocking buffer</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Standard diluent</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Detection antibodies</td>
<td>4.8 ml</td>
</tr>
</tbody>
</table>

a. Allow vial to sit at room temperature for a minimum of 5 min, not to exceed 30 min.
b. Mix by vortexing at a medium setting.
2. Bring the 10x assay buffer to room temperature (RT).
   a. Mix by inversion to ensure all salts are in solution.
   b. Prepare 1x assay buffer — dilute 1 part 10x assay buffer (60 ml) with 9 parts of dH$_2$O (540 ml).

**Test Sample Preparation**
Thaw and dilute samples within 1 hr of use. Remove any particulates by centrifugation or filtration. Avoid multiple freeze and thaw cycles.

**Dilution of Standard (1:3 Serial Dilution)**
This preparation provides sufficient volume to run duplicate standard dilution curves. Ensure that each new standard is mixed well by vortexing before proceeding to the next dilution. Change tips between each dilution.

*Note:* The product data sheet in each kit lists the most concentrated point on the standard curve (S1). Enter the values and units into Bio-Plex Manager™ software as instructed in the Read Plate section.

1. Label 8 polypropylene tubes S1 through S8. Label an additional tube Blank.
2. Transfer the reconstituted standard into the tube labeled “S1.”
3. Add the appropriate amount of the standard diluent into the labeled tubes according to Figure 3 (this will be sufficient for duplicate standard curves and blanks).
4. Prepare working standards (S2–S8) by 1:3 (threefold) serial dilution. Transfer the appropriate volume of standard into each of the labeled tubes containing standard diluent as outlined above.
5. Vortex each standard at a medium setting for 5 sec before proceeding with the next serial dilution. Change pipet tip at each dilution step.
5. Prepare Samples

The kit has sufficient reagents to run 19 fractionated samples (cytosolic and nuclear + mitochondrial fractions) in duplicate or 38 total homogenates in duplicate.

The following reagents are recommended for the preparation of fractionated samples and total homogenate:

- **Protease inhibitors** — complete, Mini; Roche Diagnostics GmbH (cat #11 836 153 001), or equivalent
- **Refrigerated centrifuge**
- **Tubes**
  a. 1.5 ml conical microcentrifuge tubes, USA Scientific (cat #1415-2500)
  b. 2 ml screw cap tubes, USA Scientific (cat #1420-8700)
- **Homogenizer**
  a. **Pestle grinder** — Kontes grinder (cat #KT749540-0000), pestle (cat #KT749520-0090), and 1.5 ml Kontes grinder snap cap tube (cat #KT749520-0090), or equivalent
b. **Homogenizer** — Bio-Gen Pro200 (cat #01-01200) with a 5 mm generator (cat #02-05075) or equivalent.

c. **Dounce homogenizer** — Fisher (cat #06-434) using tight pestle, or equivalent.

---

**Preparation of Cytosolic and Nuclear + Mitochondrial (N + M) Fractions**

**Cytosolic Fraction**

1. Prepare the cytosolic extraction buffer (CEB) and lysate dilution buffer (LDB) by adding 1 tablet each of the protease inhibitors listed above to 10 ml of each buffer. Once the tablets are in solution, store the buffers at 4°C for up to 1 week or at −20°C for up to 3 months.

2. Pre-chill all tubes and keep samples on ice during sample preparation. CEB additions and homogenization of samples are summarized in Table 5.

3. Homogenize the samples using the following guidelines (depending on sample type, additional time may be needed to ensure the sample is completely homogenized).

   a. **Tissue sample with homogenizer** — add 100 µl CEB to the 2 ml screw cap tube containing tissue and mince the tissue 10–15x using micro scissors. Add the remaining volume of CEB and

---

### Table 5. Summary of homogenization methods for cytosolic and Nuclear + Mitochondrial fractions.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Homogenization Method</th>
<th>Cytosolic Extraction Buffer (CEB) Additions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue: 20–30 mg (750 µl total volume)</td>
<td>Homogenizer</td>
<td>Add 100 µl, mince, add 650 µl, and homogenize</td>
</tr>
<tr>
<td>Tissue: &lt;20 mg (ratio of 30 µl CEB to 1 mg of tissue)</td>
<td>Homogenizer</td>
<td>Add 100 µl, mince, add remaining, and homogenize</td>
</tr>
<tr>
<td>Tissue: fine needle aspirate (350 µl total volume)</td>
<td>Homogenizer</td>
<td>Add 100 µl, mince, add 250 µl, and homogenize</td>
</tr>
<tr>
<td>Cultured cells: 5 x 10⁶ to 2 x 10⁷ (750 µl total volume)</td>
<td>Dounce homogenizer</td>
<td>Resuspend in 750 µl, incubate 45–60 min on ice, and dounce with ~40 passes (will depend on cell line)</td>
</tr>
</tbody>
</table>
homogenize the minced tissue for approximately 5 sec on medium power. Homogenized tissue samples should be free of large tissue fragments. If large fragments are visible in the sample, homogenize for an additional 5 sec. Any large fragments remaining after the additional 5 sec homogenization step should be removed before proceeding. Incubate processed samples on ice for 10 min. After incubation, transfer the sample to a prechilled 1.5 ml microcentrifuge tube.

b. **Tissue sample with pestle grinder** — add 100 µl CEB to the 1.5 ml pestle tube containing tissue sample. Grind the tissue for approximately 10 sec or until no large pieces remain. Then add the remaining volume of CEB, and remove any large fragments that did not homogenize. Incubate processed samples on ice for 10 min.

c. **Cultured Cells**
   i. Collect the cells (5 x 10⁶ to 2 x 10⁷) by centrifugation at 4°C for 5 min at 500 x g
   ii. Wash the cells in 1–2 ml of ice-cold PBS. Centrifuge at 4°C for 5 min at 500 x g. Remove supernatant.
   iii. Freeze the pellet at −80°C for a minimum of 15 min. Remove and place on ice.
   iv. Add 750 µl CEB to the pellet, resuspend the cells by pipetting up and down 10–15x, and vortex for 5 sec.
   v. Incubate the suspension on ice for 45–60 min.
   vi. Homogenize on ice using approximately 40 passes with a Dounce homogenizer.

   **Note:** The number of passes will depend on the cell line and may need to be adjusted by the end user for effective lysis.

   vii. Transfer the homogenate to a 1.5 ml microcentrifuge tube.

4. Centrifuge the homogenized sample at 4°C for 10 min at 10,000 x g.

5. Carefully remove the supernatant with a pipet and transfer to a prechilled 1.5 ml microcentrifuge tube. Measure the volume of cytosolic fraction removed, add an equal amount of LDB, and vortex. Label as cytosolic fraction.
6. Aliquot samples at appropriate volumes into labeled tubes and store at –80°C. Prepare an aliquot for protein determination. Cytosolic fractions may be stored on ice while the nuclear + mitochondrial fractions are prepared.

**Nuclear + Mitochondrial Fraction**

7. Wash the pellet from the cytosolic preparation by resuspending in 750 µl of CEB and centrifuge at 4°C for 10 min at 10,000 x g.

8. Aspirate and discard the supernatant from the tube, being careful not to disturb the pellet.

9. Resuspend the pellet in LDB. The volume of LDB needed will be the same as the total volume of CEB used in Table 5. Be sure to completely disrupt the pellet by pipet mixing. Vortex for approximately 10 sec and incubate sample on ice for 15 min, vortexing for 10 sec at each 5 min interval.

10. Centrifuge the samples at 4°C for 10 min at 10,000 x g.

11. Aspirate the supernatant and transfer to a 1.5 ml snap cap tube labeled “N + M Fraction”. Be sure not to disturb the pellet when aspirating the supernatant. Discard the pellet. Aliquot samples at appropriate volumes into labeled tubes, store at –80°C, and prepare an aliquot for protein determination.

12. Determine the protein concentration of the samples using the Bio-Rad protein assay reagent (Bio-Rad cat #500-0006) or equivalent and diluting samples 1:20 in PBS.

13. For sample analysis, bring samples to final concentrations of 250 µg/ml for Apoptosis Panel 1 and 500 µg/ml for Apoptosis Panels 2 and 3 by diluting with LDB. Additional dilutions may be required depending on sample.

**Preparation of Total Extracts**

1. Prepare the LDB by adding 1 tablet each of the protease inhibitors listed above to 10 ml of buffer. Once the tablets are in solution, store the buffer at 4°C for up to 1 week or at –20°C for up to 3 months.
2. Prechill all tubes and keep samples on ice during sample preparation. LDB additions and homogenization of samples are summarized in Table 6.

Table 6. Summary of homogenization methods for total extracts.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Homogenization Method</th>
<th>Lysate Dilution Buffer (LDB) Additions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue sample: 20–30 mg (750 µl total volume)</td>
<td>Homogenizer</td>
<td>Add 100 µl, mince, add 650 µl, and homogenize</td>
</tr>
<tr>
<td></td>
<td>Pestle grinder</td>
<td>Add 100 µl, grind, and add 650 µl</td>
</tr>
<tr>
<td>Tissue sample: &lt;20 mg (ratio of 30 µl LDB to 1 mg of tissue)</td>
<td>Homogenizer</td>
<td>Add 100 µl, mince, add remaining, and homogenize</td>
</tr>
<tr>
<td></td>
<td>Pestle grinder</td>
<td>Add 100 µl, grind, and add remaining</td>
</tr>
<tr>
<td>Tissue sample: fine needle aspirate (350 µl total volume)</td>
<td>Homogenizer</td>
<td>Add 100 µl, mince, add 250 µl, and homogenize</td>
</tr>
<tr>
<td></td>
<td>Pestle grinder</td>
<td>Add 100 µl, grind, and add 250 µl</td>
</tr>
<tr>
<td>Cultured cells: 5 x 10⁶ to 2 x 10⁷ (750 µl total volume)</td>
<td>Dounce homogenizer</td>
<td>Resuspend in 750 µl, incubate 30 min on ice, and dounce with ~40 passes (depends on cell line)</td>
</tr>
</tbody>
</table>

3. Homogenize the samples using the following guidelines (depending on sample type, additional time may be needed to ensure the sample is completely homogenized).

a. **Tissue sample with homogenizer** — add 100 µl LDB to the 2 ml screw cap tube containing tissue and mince the tissue 10–15x using micro scissors. Add the remaining volume of LDB and homogenize the minced tissue for approximately 5 sec on medium power. Homogenized tissue samples should be free of large tissue fragments. If large fragments are visible in the sample, homogenize for an additional 5 sec. Any large fragments remaining after the additional 5 sec homogenization step should be removed before proceeding. Incubate processed samples on ice for 10 min. After incubation, transfer the sample to a prechilled 1.5 ml microcentrifuge tube.

b. **Tissue sample with pestle grinder** — add 100 µl LDB to the 1.5 ml pestle tube containing tissue sample. Grind the tissue for approximately 10 sec or until no large pieces remain. Then add the remaining volume of LDB, and remove any large tissue fragments that did not homogenize. Incubate the processed samples on ice for 10 min.
c. **Cultured Cells**

i. Collect the cells \((5 \times 10^6 \text{ to } 2 \times 10^7)\) by centrifugation at \(4^\circ\text{C}\) for 5 min at 500 \(x\) g. Discard supernatant.

ii. Wash the cells in 1–2 ml of ice-cold PBS. Centrifuge at \(4^\circ\text{C}\) for 5 min at 500 \(x\) g. Discard supernatant.

iii. Add 750 µl LDB to the pellet, resuspend the cells by pipetting up and down 10–15x, and vortex for 5 sec.

iv. Incube the suspension on ice for 30 min.

v. Homogenize using with a Dounce homogenizer.

vi. Transfer the homogenate to a 1.5 ml microcentrifuge tube.

4. Centrifuge the homogenized tissue at \(4^\circ\text{C}\) for 10 min at 10,000 \(x\) g.

5. Carefully remove the supernatant with a pipet and transfer to a prechilled 1.5 ml microcentrifuge tube. Label as total extract.

6. Aliquot samples at appropriate volumes into labeled tubes and store at \(–80^\circ\text{C}\). Prepare an aliquot for protein determination.

7. For sample analysis, bring samples to final concentrations of 250 µg/ml for Bio-Plex Pro RBM Apoptosis Panel 1 and 500 µg/ml for Apoptosis Panels 2 and 3 by diluting with LDB. Additional dilutions may be required depending on sample.

6. **Run the Assay**

**Considerations**

- Bring all assay components and samples to room temperature before use
- Use calibrated pipets and pipet carefully, avoiding bubbles. Use new pipet tips for every volume transfer
- Pay close attention to vortexing, shaking, and incubation instructions. Deviation from protocol may result in low assay signal and assay variability
- Assay incubations are carried out in the dark on a shaker at \(850 \pm 50 \text{ rpm}\). Cover the plate with a plate seal and protect from light with aluminum foil
Table 7. Summary of wash options and protocols. After each assay step, select the appropriate Bio-Plex Pro wash station program or perform the appropriate manual wash step as summarized below.

<table>
<thead>
<tr>
<th>Assay Step</th>
<th>Bio-Plex Pro or Pro II Wash Station</th>
<th>Bio-Plex Pro II Wash Station</th>
<th>Handheld Magnet or Vacuum Manifold</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Magnetic Program</td>
<td>Vacuum Program</td>
<td>Manual Wash Steps</td>
</tr>
<tr>
<td>Sample incubation</td>
<td>MAG x3</td>
<td>VAC x3</td>
<td>3 x 100 μl</td>
</tr>
<tr>
<td>SA-PE incubation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Considerations When Using a Vacuum Manifold**
- After each incubation, place the filter plate on a calibrated vacuum apparatus and remove the liquid by vacuum filtration.
- To wash, add 100 μl wash buffer to each well and remove the liquid as before. Ensure that all wells are exposed to the vacuum.
- Thoroughly blot the bottom of the filter plate with a clean paper towel between each vacuum step to prevent cross contamination.
- Place the assay plate on the plastic plate holder/tray as needed.
- Before each incubation, gently cover the plate with a new plate seal. Avoid pressing down on the wells to prevent leaking from the bottom.

**Assay Protocol: Dispensing of Reagents**
1. **Add 10 μl** of blocker to all wells of the plate.
2. **Add 30 μl** of the standard, control, or sample to the appropriate well of the plate.
3. **Vortex** the capture beads at medium speed for **10–20 sec**. Add **10 μl** of the beads to all wells of the plate.
4. Cover plate with plate seal and protect from light with aluminum foil. Incubate on shaker at **850 ± 50 rpm** for **1 hr** at RT.
5. Wash the plate three times with **100 μl** 1x assay buffer.
6. **Vortex** the reconstituted detection antibodies at medium speed for **10–20 sec**. Add **40 μl** to each well.
7. Cover and incubate at 850 ± 50 rpm, as in Step 4, for 1 hr at RT. Do not aspirate after incubation.

8. Prepare the required dilution of streptavidin-PE (SA-PE) as outlined in Table 8.

Note: Volumes in the table are for an entire 96-well plate. Smaller volumes can be prepared, provided that dilution ratios are maintained.

Table 8. SA-PE dilution.

<table>
<thead>
<tr>
<th>SA-PE Dilution</th>
<th>Volume of SA-PE, µl</th>
<th>Volume of 1x Assay Buffer, µl</th>
<th>Total Volume, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>225</td>
<td>2,025</td>
<td>2,250</td>
</tr>
</tbody>
</table>

9. Add 20 µl of diluted SA-PE to the required plate wells.

10. Cover and incubate at 850 ± 50 rpm, as in Step 4, for 30 min at RT.

11. Wash the plate three times with 100 µl 1x assay buffer.

12. After the final wash, resuspend the beads in 100 µl assay buffer. Cover plate as in Step 4 and shake the plate at 850 ± 50 rpm for 30 sec.

13. Remove the plate seal and read plate at low PMT (Bio-Plex® 200), standard PMT (Bio-Plex 3D), or default settings (Bio-Plex® MAGPIX™).

7. Read Plate

Bio-Plex Manager™ software is recommended for all Bio-Plex Pro™ assay data acquisition and analysis. Instructions for Luminex xPONENT software are also included. For instructions on using other xMAP system software packages, contact Bio-Rad Technical Support or your regional Bio-Rad field applications specialist.
Prepare Protocol in Bio-Plex Manager Software Version 6.0 and Higher

The protocol should be prepared in advance so that the plate is read as soon as the experiment is complete.

A protocol file specifies the analytes used in the reading, the plate wells to be read, sample information, the values of standards and controls, and instrument settings. Protocols may be obtained from within Bio-Plex Manager software version 6.1 or created from the File menu.

To create a new protocol, select **File**, then **New** from the main menu. Locate and follow the steps under **Protocol Settings**.

1. Click **Describe Protocol** and enter information about the assay (optional).
2. Click **Select Analytes** and create a new panel.
   a. Click **Add Panel** in the Select Analytes toolbar. Enter a new panel name. Select **Bio-Plex Pro Assay Magnetic** from the assay dropdown menu. If using Bio-Plex Manager version 5.0 or lower, select **MagPlex** from the assay dropdown menu.
   b. Click **Add**. Enter the bead region number and name for the first analyte. Click **Add Continue** to repeat for each analyte in the assay. Refer to the bead regions in parentheses ( ) listed on the peel-off label provided with the standards.

   For reference, bead regions for the individual assays are listed in Table 8.
   c. Click **Add** when the last analyte has been added and click **OK** to save the new panel.
   d. Highlight analytes from the **Available list** (left) and move to the **Selected** list (right) using the **Add** button. To move all analytes at once, simply click **Add All**.
e. If some of the analytes need to be removed from the Selected list, highlight them and select **Remove**. If desired, it is possible to rename the panel by clicking on **Rename Panel** and entering a new panel name.

<table>
<thead>
<tr>
<th>Panel 1</th>
<th>Bak (74)</th>
<th>Bax (27)</th>
<th>Lamin B (14)</th>
<th>Smac (19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panel 2</td>
<td>Bad (73)</td>
<td>Bcl-2/Bax (42)</td>
<td>Bcl-xL (22)</td>
<td>Bim (12)</td>
</tr>
<tr>
<td>Active</td>
<td>caspase (57)</td>
<td>Bcl-xL/Bak (47)</td>
<td>Mcl-1/Bak (54)</td>
<td>Survivin (20)</td>
</tr>
</tbody>
</table>

3. Click **Format Plate** and format the plate according to the plate layout template (located at the back of the manual) created for the assay. To modify the plate layout, follow the steps below (see Figure 4).

a. Select the **Plate Formatting** tab.

b. Select the standards icon  and drag the cursor over all the wells that contain standards. Repeat this process for blanks , controls , and samples .

![Fig. 4. Plate formatting.](image-url)
4. Click **Enter Standards Info** in the Protocol Settings bar.
   a. Enter the highest concentration of each analyte in the top row (labeled S1) of the table. S1 concentration information is listed in the product data sheet.
   b. Enter a dilution factor of 3 and click **Calculate**. The concentrations for each standard point will be populated for all analytes in the table.
   c. Optional: enter the lot number of the vial of standards into the **Standard Lot** box and click **Save**.

5. Click **Enter Controls Info**.
   a. For user-specified controls, select an analyte from the dropdown menu, then enter a description and concentration. Repeat for each additional analyte in the assay.
   b. For the kit controls supplied, format the appropriate wells as controls and enter descriptions, but leave the concentrations blank. Alternatively, the controls can be formatted as samples with clear descriptions such as “quality control.” In any case, the expected control ranges provided on the product data sheet are not entered into Bio-Plex Manager software version 6.1 and earlier.

6. Click **Enter Sample Info** — enter sample information and the appropriate dilution factor.

7. Click **Run Protocol** — confirm that the assay settings follow Table 10.

**Table 10. Read the plate using the appropriate instrument settings.**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>RP1 (PMT)</th>
<th>DD Gates</th>
<th>Bead Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Plex 100, 200*</td>
<td>Low</td>
<td>5,000 (low), 25,000 (high)</td>
<td>50</td>
</tr>
<tr>
<td>Bio-Plex 3D*</td>
<td>Standard</td>
<td>Select MagPlex beads</td>
<td>50</td>
</tr>
<tr>
<td>Bio-Plex® MAGPIX™*</td>
<td>N/A, use default instrument settings</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A similar Luminex-based system may be used.
a. Confirm that data acquisition is set to **50 beads per region**.

b. In Bio-Plex Manager software prior to 6.1, go to Advanced Settings, confirm that the bead map is set to **100 region**, the sample size is set to **50 µl**, and the DD gates are set to **5,000 (Low) and 25,000 (High)**. In Bio-Plex Manager software versions 4.0, 4.1, 4.1.1, and 5.0, check **Override Gates** and set the DD gate values as indicated.

Select **Start**, name and save the .rbx file, and begin data acquisition. The Run Protocol pop-up screen will appear. Click **Eject/Retract** to eject the plate carrier.

**Acquire Data**

1. Shake the assay plate at **850 ± 50 rpm** for **30 sec**, and visually inspect the plate to ensure that the assay wells are filled with buffer. Slowly remove the sealing tape and any plate cover before placing the plate on the plate carrier.

2. Click **Run Protocol** — on the pop-up screen, select **Load Plate** and click **OK** to start acquiring data.

3. Use the **Wash Between Plates** command after every plate run to reduce the possibility of clogging the instrument.

4. If acquiring data from more than one plate, empty the waste bottle and refill the sheath bottle after each plate (if HTF are not present). Select **Wash Between Plates** and follow the instructions. Then repeat the Prepare Protocol and Acquire Data instructions.

5. When data acquisition is complete, select **Shut Down** and follow the instructions.
Data Analysis

Quality Controls
If the quality controls were run in the assay plate, open the results (.rbx) file, click Report Table, and locate the control wells. Compare the observed concentrations against the lot-specific control ranges in the product data sheet.

**Note:** Expected control ranges are provided for reference and should be used as general guidelines. Actual results may vary for some operators. If the controls do not fall within the expected ranges, please refer to the troubleshooting section for possible causes and solutions.

Removing Outliers
Outliers are identified as standard data points that do not meet accuracy or precision requirements and should be considered invalid when performing curve fitting. As such, they should be removed to generate a more realistic and accurate standard curve. This may result in an extended assay working range and allow quantitation of samples that might otherwise be considered out of range (OOR).

In Bio-Plex Manager software version 6.0 and higher, outliers can be automatically removed by selecting the Optimize button in the Standard Curve window. In earlier versions of the software, outliers can also be manually selected in the Report Table. Visit online Help to learn more about the standard curve optimizer feature and how outliers are determined.

Previous Versions of Bio-Plex Manager Software
For instructions on using previous versions of Bio-Plex Manager software, please contact Bio-Rad Technical Support.
Luminex xPONENT Software

Luminex xPONENT software may be used to analyze Bio-Plex assays. Although guidelines are provided here, consult the xPONENT software manual for more details. Perform a system initialization with Luminex’s calibration and performance verification kit, as directed by Luminex. Select Batches to set up the protocol and follow the information under Settings.

Note: The instrument settings described below apply to Luminex 100/200 and FLEXMAP 3D or Bio-Plex 3D instruments. For the Bio-Plex MAGPIX reader, use the default instrument settings.

1. Select MagPlex as the bead type for magnetic beads, which automatically sets the DD gates.
2. Volume = 50 µl
3. Low PMT (Standard PMT).
4. Plate name: 96-well plate.
5. Analysis type: Quantitative; 5PL Curve Fit.

Select Analytes to set up the panel.
1. Enter “ng/ml” in the Units field.
2. Enter 50 in the Count field.
3. Select the bead region and enter the analyte name.
4. Click Apply all for Units and Count.

Select Stds andCtrls.
1. Enter standard concentrations, lot number, dilution factor, and other information, as applicable.

After the assay is complete, select Results, then select Saved Batches.
# Troubleshooting Guide

This troubleshooting guide addresses problems that may be encountered with Bio-Plex Pro™ RBM assays. If you experience any of the problems listed below, review the possible causes and solutions provided. Poor assay performance may also be due to the Bio-Plex® suspension array reader. To eliminate this possibility, use the validation kit to validate all the key functions of the array reader and to assist in determining whether or not the array reader is functioning properly.

## Possible Causes

### High Inter-Assay CV

- Standards were not reconstituted consistently between assays
- Reconstituted standards and diluted samples were not stored properly
- Bottom of filter plate not dry

## Possible Solutions

- Incubate the reconstituted standards for the recommended time period. Always be consistent with the incubation time and temperature.
- Diluted samples should be prepared on ice as instructed. Prior to plating, the reconstituted standards and diluted samples should be equilibrated to room temperature.
- Dry the bottom of the filter plate with absorbent paper towel (preferably lint-free) to prevent cross-well contamination.
Possible Causes

**High Intra-Assay CV**
- Improper pipetting technique

**Possible Solutions**
- Pipet carefully when adding standards, samples, detection antibodies, and streptavidin-PE, especially when using a multichannel pipet. Use a calibrated pipet. Change pipet tip after every volume transfer.

- All reagents and assay components should be equilibrated to room temperature prior to pipetting.

- During the wash steps, be careful not to splash buffer from one well to another. Be sure that the wells are filtered completely and that no residual volume remains. Ensure that the microplate shaker setting is not too high. Reduce the microplate shaker speed to minimize splashing.

- Sample pipetting across the entire plate should take less than 4 min. Reagent pipetting across the entire plate should take less than 1 min.

**Low Bead Count**
- Beads clumped in multiplex bead stock tube

**Possible Solutions**
- Vortex for 30 sec at medium speed before aliquoting beads.
Possible Causes | Possible Solutions
--- | ---
**Low Bead Count**
Vacuum on for too long when aspirating buffer from wells | Do not apply vacuum to the filter plate for longer than 10 sec after the buffer is completely drained from each well.

Reader is clogged | Refer to the troubleshooting guide in the Bio-Plex system hardware instruction manual (bulletin #10005042).

**Low Signal or Poor Sensitivity**
Standards reconstituted incorrectly | Follow the standard preparation instructions carefully.

Detection antibody or streptavidin-PE prepared incorrectly | Check your calculations and be careful to add the correct volumes.

**High Background Signal**
Incorrect buffer was used (for example, assay buffer used to dilute standards) | Use standard diluent to dilute standards.

Accidentally spiked blank wells | Do not add any antigens to the blank wells.

Detection antibodies or streptavidin-PE incubated too long | Follow the procedure incubation time precisely.
<table>
<thead>
<tr>
<th>Possible Causes</th>
<th>Possible Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor Recovery</td>
<td></td>
</tr>
<tr>
<td>Expired Bio-Plex reagents were used</td>
<td>Check that reagents have not expired. Use new or nonexpired components.</td>
</tr>
<tr>
<td>Incorrect amounts of components were added</td>
<td>Check your calculations and be careful to add the correct volumes.</td>
</tr>
<tr>
<td>Microplate shaker set to an incorrect speed</td>
<td>Check the microplate shaker speed and use the recommended setting. Setting the speed too high may cause splashing and contamination. Use the recommended plate shaker.</td>
</tr>
<tr>
<td>High end saturation of the standard curve</td>
<td>Make sure that correct shaker speed and incubation times are used. Remove S1 for data analysis if needed.</td>
</tr>
<tr>
<td>Controls do not fall within expected ranges</td>
<td>Make sure that the vial of controls is reconstituted at the same time as standards and in the correct diluent. Incubate for times indicated.</td>
</tr>
<tr>
<td>Improper pipetting technique</td>
<td>Pipet carefully when adding standards, samples, detection antibodies, and streptavidin-PE, especially when using a multichannel pipet. Use a calibrated pipet. Change pipet tip after every volume transfer.</td>
</tr>
</tbody>
</table>
Possible Causes

Impact of Sample Matrix

Negative MFI values in samples

Possible Solutions

If samples contain little or no analyte, negative values observed may be due to statistical variation. If assay drift is suspected, retest the samples by positioning them next to the standards. If contamination of standards is suspected, check the standard replicate value and be careful when adding samples to the wells. Matrix effects could also produce negative sample values.

Bio-Plex Manager™ software automatically subtracts the blank (B) MFI value from all other assay wells. While this has no impact on observed concentrations of samples within the assay working range, it may result in a negative MFI value if the blank’s MFI value is greater than either the standard or the sample value. If this is undesirable, then reformat the blank wells as sample (X) or control (C) in the protocol or results file.
Plate Layout Template
Safety Considerations

Eye protection and gloves are recommended when using these products. Consult the MSDS for additional information. The Bio-Plex Pro™ assays contain components of animal origin. This material should be handled as if capable of transmitting infectious agents. Use universal precautions. These components should be handled at Biosafety Level 2 containment (U.S. government publication: Biosafety in Microbiological and Biomedical Laboratories (CDC 1999)).

Legal Notices

Bio-Plex Pro RBM kits are manufactured by Myriad RBM.

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The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.
### Ordering Information

Detailed ordering information can be found at [www.bio-rad.com/bio-plex](http://www.bio-rad.com/bio-plex).

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Premixed 1 x 96-Well All-In-One Multiplex Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Includes premixed magnetic capture beads, premixed detection antibodies, standards mix, 2-level controls, blocking buffer, standard diluent, lysate dilution buffer (LDB), cytosolic extraction buffer (CEB), 10x assay buffer, 10x streptavidin-PE, 96-well flat bottom plate, plate seals, and instructions.</td>
</tr>
<tr>
<td>171-WAR1CK</td>
<td>Bio-Plex Pro RBM Apoptosis Panel 1, 1 x 96</td>
</tr>
<tr>
<td>171-WAR2CK</td>
<td>Bio-Plex Pro RBM Apoptosis Panel 2, 1 x 96</td>
</tr>
<tr>
<td>171-WAR3CK</td>
<td>Bio-Plex Pro RBM Apoptosis Panel 3, 1 x 96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Wash Stations and Accessories</th>
</tr>
</thead>
<tbody>
<tr>
<td>300-34376</td>
<td>Bio-Plex Pro Wash Station, includes magnetic plate carrier, waste bottle, 2 buffer bottles</td>
</tr>
<tr>
<td>300-34377</td>
<td>Bio-Plex Pro II Wash Station, includes magnetic plate carrier, vacuum manifold plate carrier, waste bottle, 2 buffer bottles</td>
</tr>
<tr>
<td>171-020100</td>
<td>Bio-Plex Handheld Magnetic Washer, includes magnetic washer and adjustment hex tools for use in manual wash steps for all Bio-Plex magnetic assays</td>
</tr>
<tr>
<td>171-025001</td>
<td>Bio-Plex Pro Flat Bottom Plates, 40 x 96-well plates</td>
</tr>
<tr>
<td>171-304500</td>
<td>Bio-Plex Wash Buffer, 1.5 L</td>
</tr>
<tr>
<td>171-304502</td>
<td>Filter plate, pkg of 1, 96-well plate with clear plastic lid and tray, for Bio-Plex assays using the vacuum wash method, sealing tape not included</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Software</th>
</tr>
</thead>
<tbody>
<tr>
<td>171-001510</td>
<td>Bio-Plex Data Pro™ with Bio-Plex Manager Software (5 seats), for multi-experiment analysis and advanced data visualization</td>
</tr>
<tr>
<td>171-001513</td>
<td>Bio-Plex Data Pro Software (5 seats), for multi-experiment analysis and advanced data visualization</td>
</tr>
<tr>
<td>171-001523</td>
<td>Bio-Plex Data Pro Plus Software, contains all the features of Bio-Plex Data Pro software with added visualization, sharing, and analysis functionality</td>
</tr>
<tr>
<td>171-STND01</td>
<td>Bio-Plex Manager Software (1 seat), for instrument data evaluation and optimization</td>
</tr>
</tbody>
</table>