

Attune™ Performance Tracking Beads *for use with Attune™ Acoustic Focusing Cytometer*

Catalog no. 4449754

Table 1. Contents and storage information.

Material	Amount	Composition	Storage*	Stability
Attune™ Performance Tracking Beads consist of equal concentrations of intensity level 1, 2, 3, and 4 beads supplied in a stream-tip dropper bottle	3 mL	5 × 10 ⁶ beads/mL supplied in phosphate buffered saline (PBS) with Tween® 20 and 2 mM sodium azide	<ul style="list-style-type: none"> • 2–8°C • Protect from light • DO NOT FREEZE 	When stored as directed the product is stable for 1 year.

Number of measurements: Each vial contains beads sufficient for approximately 50 daily measurements or 50 baseline definitions based on the protocol below.

Introduction

Applied Biosystems' Attune™ Performance Tracking Beads (PT Beads) are designed for research use only with Applied Biosystems Attune™ Cytometric Software on the Attune™ Acoustic Focusing Cytometer. The beads allow the software to automatically characterize, track, and report performance measurements of Attune™ Acoustic Focusing Cytometer.¹

Each vial of PT Beads contains a mixture of equal concentrations of beads of four fluorescence emission intensities (intensity levels 1, 2, 3, and 4). The beads are used to define a baseline and conduct daily measurements of the cytometer. Each vial contains beads sufficient for approximately 50 daily measurements or 50 baseline definitions.

The intensity level 1 beads in Attune™ Performance Tracking Beads are 2.4 µm in nominal diameter. The intensity level 2, 3, and 4 beads are 3.2 µm in nominal diameter, and stained with a combination of multiple fluorophores that can be excited by the lasers used in the Attune™ Acoustic Focusing Cytometer and emit fluorescence signals at designated levels to all the channels in the instrument.

Baseline Calculation

PT Beads may be used to define a cytometer baseline,² which is performed anytime a new lot of PT Beads are used, or after any major maintenance is performed on the instrument. Diluted beads are run on the acoustic focusing cytometer using the Attune™ Cytometric Software. The percent half-peak coefficient of variation (%HPCV) of the intensity level 4 bead is recorded. Using assigned MESF values for each fluorescent bead, the relative quantum efficiency (rQ) and relative background (rB) is calculated for each channel, and the linear regression is calculated and recorded. The laser delay setting is also automatically calculated.

Performance Tracking

Once baseline values are defined, the PT Beads are used to run daily performance measurements to track the daily performance of the cytometer. We recommend that you run the performance test at least once per day after the instrument start up is initiated. The intensity level 4 bead is placed in the target channel and the PMT voltage is recorded, and then compared to the previous PMT voltage to calculate the delta. The %HPCV of the bead is recorded. Using assigned MESF values for each fluorescent bead, the relative quantum efficiency (rQ) and relative background (rB) is calculated for each channel, and the linear regression is calculated and recorded. The laser delay setting is also automatically calculated. Levey-Jennings charts provide a visual to track the %HPCV and PMT voltage to check for shifts and trends.

Before Starting

Materials Required but Not Provided

- Disposable 12 × 75 mm tubes, 1.5 mL microcentrifuge tubes, or equivalent
- Vortex mixer, optional
- Attune™ Focusing Fluid or Phosphate buffered saline (PBS) for use as a diluent
- Attune™ Acoustic Focusing Cytometer manual for operating instructions

Caution

Attune™ Performance Tracking Beads solution contains 0.05% sodium azide as a preservative. Sodium azide is an extremely toxic and dangerous compound particularly when combined with acids or metals. Properly dispose of solutions containing sodium azide.

Experimental Protocols

Running a New Lot of Beads

Before using a new lot of beads:

- 1.1 Go to www.appliedbiosystems.com/attunecytometer.
- 1.2 Import the lot-specific data file (refer to the Attune™ user's manual for instructions on how to import the lot-specific information into Attune™ Cytometric Software).
- 1.3 To verify the lot number of the PT Beads, look at the first six digits printed on the lot number (L/N) field on the label. Ignore the alpha character at the end of the lot number.
- 1.4 Refer to the Attune™ Acoustic Focusing Cytometer manual for details on running a new lot of beads on the instrument.

Preparing Attune™ Performance Tracking Beads in Tubes

Brief instructions for preparing the beads are described below. For detailed instructions and troubleshooting, refer to the Attune™ Acoustic Focusing Cytometer manual supplied with the instrument or available for download at www.appliedbiosystems.com.

Optimization of cytometer settings for applications using stained biological samples may be required following the cytometer setup.

Prepare the Attune™ Performance Tracking Bead suspension immediately before use.

- 2.1 Label a flow tube, or a standard 12 × 75 mm test tube, or a 1.5 mL microcentrifuge tube.
- 2.2 Mix the bead vial by gentle inversion or gentle vortexing.
- 2.3 Prepare the beads suspension for defining baseline or running daily measurements by adding the following to the labeled tube:

Diluent	1 mL
PT Beads	1 drop

Note: Attune™ Performance Tracking Beads can be diluted in Attune™ Focusing Fluid or PBS. For consistent results, always use the same diluent and sample delivery device to run the Attune™ Performance Tracking Beads.

- 2.4 Mix the bead suspension by gentle inversion or vortexing.
- 2.5 Refer to the Attune™ Acoustic Focusing Cytometer manual for details on running a performance measurement.

Note: If not using immediately, store the diluted bead suspension at 2°C to 25°C, **protected from light**, for no more than 4 hours.

References

1. Fundamentals of Acoustic Cytometry In: Current Protocols in Cytometry 1.22.1 (2009); 2. Standardization and quantitation in flow cytometry. In: Methods Cell Biol. 63, 300 (2001).

Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
4449754	Attune™ performance tracking beads *for use with the Attune™ Acoustic Focusing Cytometer* *intensity levels 1, 2, 3, 4* *5 × 10 ⁶ beads/mL*	3 mL
Related Products		
4449790	Attune™ 1X Focusing Fluid	1 L
4449791	Attune™ 1X Focusing Fluid	6 × 1 L
4449792	Attune™ 10X Focusing Fluid	1 L
4449755	Attune™ Wash Solution	500 mL

Contact Information

Applied Biosystems

850 Lincoln Centre Drive
Foster City, CA 94404
Phone: (650) 638-5800
Toll free: (800) 345-5224

Technical Resources and Support:

For the latest technical resources and support information for all locations, visit our website at www.appliedbiosystems.com/support

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