

Attune™ Performance Tracking Beads

Catalog Number 4449754

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Rev. A.0

Table 1 Contents and storage

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 performance measurements or 50 baseline definitions based on the protocol below.				

Product information

Invitrogen™ Attune™ Performance Tracking Beads (PT Beads) are designed for use with the Attune™ and Attune™ NxT Acoustic Focusing Cytometers. The beads allow the instrument software to automatically characterize, track, and report performance measurements of the 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 performance 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, while the intensity level 2, 3, and 4 beads are 3.2 µm in nominal diameter. The beads are stained with a combination of multiple fluorophores that can be excited by the lasers used in the Attune™ and Attune™ NxT Acoustic Focusing Cytometers 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 instrument software, and 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) are calculated for each channel, and the linear regression is calculated and recorded. The laser delay setting is also automatically calculated.

Performance tracking After the 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 you begin

Materials required but not provided

- Disposable 12 × 75-mm tubes, 1.5-mL microcentrifuge tubes, or equivalent
- Phosphate buffered saline (PBS) or deionized water for use as a diluent
- *Optional:* Vortex mixer

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.

Methods

Attune™ Performance Tracking Beads are designed for use with the classic Attune™ Acoustic Focusing Cytometer and the new generation Attune™ NxT Acoustic Focusing Cytometer.

Run a new lot of beads Before using a new lot of beads:

- 1.1 Go to Attune™ Performance Tracking Beads product page on our website (www.thermofisher.com/order/catalog/product/4449754).
- 1.2 Import the lot-specific data file (refer to the instrument user guide for instructions on how to import the lot-specific information into the 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 numeric characters 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.

Prepare Attune™ Performance Tracking Beads in tubes

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

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

IMPORTANT! Prepare the Attune™ Performance Tracking Bead suspension immediately before use. Performance test data may be invalid or the test may fail if using beads that have been diluted and stored for over 4 hours.

- 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 beads thoroughly by inverting or vortexing the vial.
- 2.3 Prepare the beads suspension for defining baseline or running daily measurements by adding the following components to the labeled tube.

IMPORTANT! Do not prepare the Attune™ Performance Tracking Beads at a dilution different than described below. Preparation of the beads at incorrect concentrations may result in failure of baseline and/or performance test.

	Attune™ Cytometer	Attune™ NxT Cytometer
Diluent*	1 mL	2 mL
PT Beads	1 drop	3 drops

* PBS or Attune™ Focusing Fluid.

Note: Attune™ Performance Tracking Beads can be diluted in PBS or Attune™ Focusing Fluid. 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 inversion or vortexing.

IMPORTANT! Attune™ Performance Tracking Beads must be thoroughly mixed with the diluent. Failure to adequately mix beads in diluent will lead to performance test failure.

- 2.5 Refer to the Attune™ or Attune™ NxT Acoustic Focusing Cytometer user guide for details on running the 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.

- 2.6 After the completion of the performance test, ensure that the system is washed using the recommended wash protocol:
 - **Attune™ Cytometer:** Prepare a fresh dilution of 10% bleach and run 1.5 mL as a sample at a collection rate of 1000 µL/minute. Immediately follow by running 1.5 mL of deionized water at a collection rate of 1000 µL/minute.
 - **Attune™ NxT Cytometer:** Prepare a fresh dilution of 10% bleach and perform the SIP sanitize function.

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).

Ordering information

Cat. No.	Product name	Amount
4449754	Attune™ Performance Tracking Beads	3 mL
Related products		
4488621	Attune™ Focusing Fluid (1X)	1 L
4449791	Attune™ Focusing Fluid (1X)	6 × 1 L
A24904	Attune™ Focusing Fluid (1X)	10 L
A24974	Attune™ Wash Solution	250 mL
A24975	Attune™ Shutdown Solution (1X)	250 mL
A10496	Attune™ Debubble Solution	50 mL

Documentation and support

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining support

For the latest services and support information for all locations, go to thermofisher.com/support.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (thermofisher.com/support)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS

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Revision history: Pub. No. MAN0002636

Revision	Date	Description
A	October 2016	Update branding and URLs, add dilution instructions for use with Attune™ NxT Cytometer.
1	February 2010	New document.

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