Countable Labs.

ANCORA *NPM1* MUTATION ASSAY (RNA)

Standardize *NPM1* quantitation with direct RNA counting

NPM1 Types A, B, and D, plus *ABL1 reference*, in one reaction

NPM1 mutation measuring has typically forced a choice between the sensitivity of RNA-level detection with NGS and a validated DNA-based qPCR assay using a standard curve. Standard curves introduce run-to-run variability and add a layer between the sample and the result. Labs need a streamlined way to identify common *NPM1* mutation types and quantify their abundance from extracted RNA.

The Ancora *NPM1* RNA assay detects and quantifies mutation Types A, B, and D alongside an *ABL1* reference in one reaction. Powered by Countable PCR, the assay isolates molecules across 30 million compartments, and delivers absolute molecular counts without per-run standard curves. Automated reporting identifies the detected mutation type and reports mutant transcript abundance as a mut*NPM1/ABL1* ratio (%).

Key Highlights

RNA-driven sensitivity: Detect lower-level *NPM1* mutant transcripts directly from RNA.

Multiplex variant detection: Quantify *NPM1* Types A, B, and D alongside an *ABL1* reference in one reaction.

Direct molecular counting: Isolate molecules across 30 million compartments for absolute quantification without per-run standard curves.

Simplified workflow, fast turnaround: One PCR workflow takes extracted RNA straight to absolute molecular counts.

Maximized sample utility: Consolidate variant detection and quantification in a single-tube workflow with up to 30 µL volume input, eliminating separate, variant specific reflex reactions for Types A, B, and D.



RESULTS SUMMARY — %RATIO

- *%Ratio is calculated by mutNPM1 / ABL1 counts per 50 µL.*

mut*NPM1* detected	Sample ID	Sample quality	%Ratio	mut*NPM1* counts per 50 µL	*ABL1* counts per 50 µL
mut*NPM1* Type A	214743_02	Pass	6.906	58,623	848,906
mut*NPM1* Type A	21406F_01	Pass	0.005%	39	809,434
mut*NPM1* Type B	21406F_02	Pass	0.003%	24	848,498
mut*NPM1* Type B	21406F_03	Pass	0.004%	36	827,454
mut*NPM1* Type D	214741_02	Pass	0.001%	10	836,830
mut*NPM1* Type D	214183_01	Pass	62.77%	510,527	813,267

Figure 1. End-to-end Ancora *NPM1* Mutation Assay solution. The kit includes all reagents, controls, and consumables needed to run the assay. Extracted total RNA undergoes reverse transcription and amplification, followed by imaging and automated analysis using Countable Control Software. The report identifies the detected *NPM1* mutation type and provides mutant *NPM1* and *ABL1* transcript counts per 50 µL, with mutant transcript abundance reported as a mut*NPM1/ABL1* ratio (%).

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Linearity across Types A, B, and D

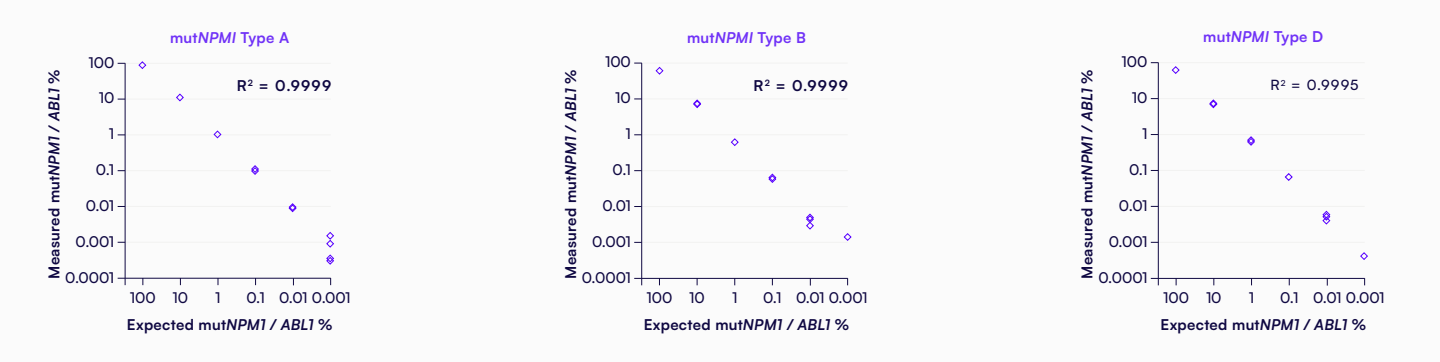


Figure 2. Linearity across *NPM1* Types A, B, and D. Measured mut*NPM1*/*ABL1* ratios (%) tracked expected ratios across five orders of magnitude ($R^2 > 0.999$).

Variants covered by *NPM1* panel

Table 1. Multiplex detection of *NPM1* Types A, B, and D and *ABL1* reference. Each target is assigned a distinct optical signature, enabling identification and absolute counting in one reaction.

Variant	Frequency in *de novo* AML
Type A	~72%
Type B	~12%
Type D	~4%

ABL1 used as reference target
Source: https://pmc.ncbi.nlm.nih.gov/articles/PMC5843774/

Performance

The broad dynamic range of the Countable PCR system lets the Ancora *NPM1* Mutation Assay count rare mutant transcripts against abundant *ABL1* reference transcripts. Each molecule amplifies in its own compartment, preventing abundant reference targets from outcompeting low-level *NPM1* transcripts. The system supports a 6-log dynamic range. Because every molecule is counted directly, quantification is absolute and no standard curve is needed.

Table 2. Dynamic input flexibility enables limits of detection (LoD) down to 0.001% mut*NPM1*/ABL1 at 1M *ABL1* copies.

ABL1 Expression Counts	Limit of Detection (%)
100,000	0.01%
500,000	0.002%
1,000,000	0.001%

Sample requirements:

- Sample type: Extracted total RNA (from EDTA whole blood or bone marrow aspirate).
- Minimum input: ≥20 ng RNA (for mutation type determination).
- High sensitivity input: ≥100 ng RNA and *ABL1* counts close to 1,000,000 (depending on sample availability and desired sensitivity level).

Ordering

SKU	Kit Name	Description
BK0007	Ancora *NPM1* (RNA) Bundle	This complete 48-reaction solution includes two kits and a software module: (1) One tube multiplexed 4-color assay that detects the three most common *NPM1* mutations (Type A, Type B, and Type D) against reference gene *ABL1* in a single RNA-input reaction with Imaging Control (48 reactions) included; (2) Countable PCR consumables and matrix reagents; and (3) a software module for data collection and reporting.

Required equipment: This assay is designed for use exclusively with the Countable System and its compatible equipment.

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To learn more, visit countablelabs.com or contact support at support@countablelabs.com.

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