

NPM1 One-Step MRD Kit

Real-Time PCR assay

- Detects NPM1 Type A, B, and D mutant transcripts
- One-step cDNA synthesis
- 32 tests per kit
- Available as RUO

INTENDED USE:

The NPM1 One-Step Detection MRD (minimal residual disease) Kit is intended for the quantitative detection of NPM1 Type A, Type B, and Type D transcripts in RNA isolated from peripheral blood or bone marrow samples. This kit is intended for research use only. Not for use in diagnostic procedures.

FOR USE WITH:

- Applied Biosystems – QuantStudio (all models)
- Bio-Rad – CFX96 and CFX96 Opus
- Qiagen – RotorGene Q
- Roche – LightCycler 480, Cobas 4800, Cobas z480

PACKAGE CONTENTS

This product contains the following materials sufficient for 32 reactions, including samples and controls.

Description	Tubes
One-Step QRT-PCR Reaction Mix (5X)	1
Manganese Acetate	1
NPM1 Detection Primer Mix (A, B, D)	3
NPM1 Type A Positive Control Mix	1
NPM1 Type B Positive Control Mix	1
NPM1 Type D Positive Control Mix	1

HANDLING AND STORAGE

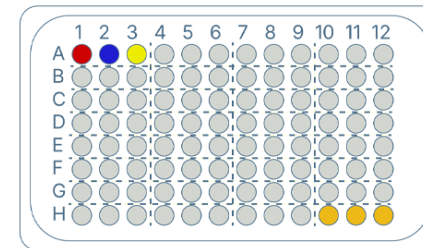
This product is shipped on frozen ice packs and may be thawed upon arrival. Do not ship on dry ice. The contents of the shipment should be frozen at -25°C to -15°C immediately upon receipt. Do not store the kit or any of its components below -25°C as it will adversely affect the performance of the assay.

- Store all unopened components in original containers.
- Primer/probe mixes should be protected from light at all times to prevent photo bleaching of the fluorescent dyes.
- Centrifuge the tubes before opening.

PLATE SETUP

A single 96-well PCR plate can accommodate up to 30 unknown samples for each reaction, a positive control mix and a no template control (NTC) for each reaction A, B, and D.

- PC-A • PC-D • NTC
- PC-B • Samples



PRINCIPLE OF THE ASSAY

Acute myeloid leukemia (AML) has a relatively low number of genomic alterations compared to other cancers. A mutation in nucleophosmin (NPM1) is one of the most commonly detected genomic alterations in AML, therefore it can be used as an indicator for newly diagnosed AML and as a leukemia-specific marker of minimal residual disease (MRD). The NPM1 quantitative MRD assay is a one-step reverse transcription and qPCR assay that detects the presence of Type A, B and D transcripts in RNA isolated from peripheral blood or bone marrow samples. The assay works by amplifying mutant-specific sequences and a FAM-labeled hydrolysis probe to detect distinct NPM1 mutations in three separate reactions. Each reaction also contains a primer set and CIV-550 (VIC equivalent)-labeled probe to detect the amplification of NPM1 as an internal control. In addition, a quantitative control is supplied to calculate percent NPM1 mutant gene expression relative to total NPM1 gene expression. Using the recommended sample input, an assay sensitivity of 5-log reduction or 0.001% can be achieved. This kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR with results in less than 2 hours.

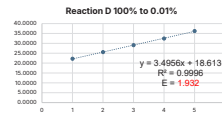
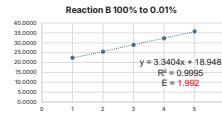
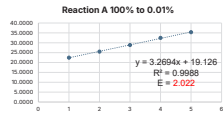
- This assay has a linear dynamic range of 5 logs.
- The accuracy of this assay greatly depends on the quality and quantity of the input RNA. Centrifugation protocol and isolation method have an impact on RNA quality and yield, refer to kit manufacturer's instructions for details.
- The performance of this assay has not been established in the presence of rare polymorphisms.
- The performance of this assay has not been established in the presence of other mutations.
- The input recommendations in this assay are based on fluorometric analysis. The performance of this assay has not been established with other quantification methods. Input requirements may require adjustment.

ASSAY PERFORMANCE

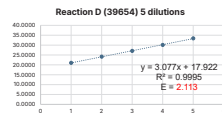
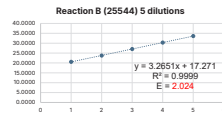
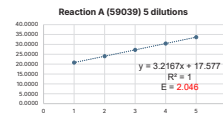
Reaction Efficiency

Five concentration levels spanning 100% to 0.01% of clinical samples confirmed for NPM1 Type A, B, and D mutations were tested in triplicate. Reaction efficiency and linearity were evaluated across the tested concentration range.

NPM1 One-Step MRD Kit

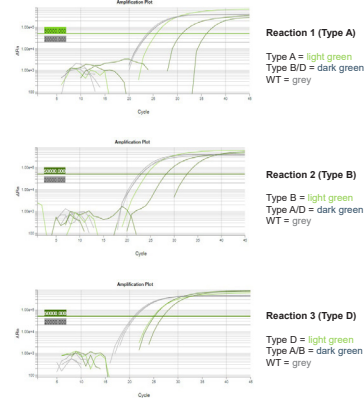


NPM1 Wild-Type (VIC)



Cross Reactivity

NPM1 Type A, B, and D mutations are differentiated using three mutation-specific reaction mixes. Mutation type is determined based on the FAM Ct pattern across the three reactions.



NPM1 Mutation	Mutation-Specific Criteria
Type A	Earliest FAM Ct value is in reaction A AND the difference in FAM Ct values across reactions is greater than 5.
Type B	Earliest FAM Ct value is in reaction B AND the difference in FAM Ct values across reactions is greater than 5.
Type D	The difference in FAM Ct values across the reactions is less than 5.

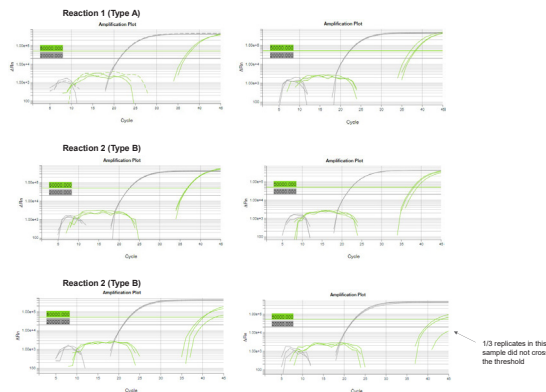
Analytical Specificity

Twelve non-mutated clinical samples were tested in singlet across two runs to evaluate assay specificity.

Limit of Detection

Detection of NPM1 Type A, B, and D mutations was evaluated using clinical samples diluted with NPM1 wild-type RNA.

0.001% NPM1 mutant / 5.0-log reduction was evaluated at an RNA input of 75 ng per reaction.



Accuracy

Thirty-six clinical samples, including 24 samples with confirmed NPM1 mutations and 12 NPM1 wild-type samples, were tested in singlet across two runs. Results were compared with clinical laboratory NGS results.

100% concordance for NPM1 mutation status was observed between the NPM1 One-Step Detection MRD Kit and NGS results.

Accession ID	Clinical Lab NGS Results		NPM1-QRT32
	NPM1 Insertion	NPM1 Type	NPM1 Type
59039	CTCTG	A	A
72629	TCTGC	D	D
53249	CTCTG	A	A
99728	CTCTG	A	A
99792	CTCTG	A	A
66231	CTCTG	A	A
71112	CTCTG	A	A
01728	CTCTG	A	A
25544	CTGCA	B	B
39654	TCTGC	D	D
78645	CTCTG	A	A
99374	TGCCG	Km	Non-ABD Type NPM1 mutation

Accession ID	Clinical Lab NGS Results		NPM1-QRT32
	NPM1 Insertion	NPM1 Type	NPM1 Type
09875	CTCTG	A	A
16549	CTGCA	B	B
23786	CTCTG	A	A
24082	CTCTG	A	A
25208	Negative	WT	WT
26154	CTCTG	A	A
35953	Negative	WT	WT
38426	Negative	WT	WT
45111	CTCTG	A	A
45516	Negative	WT	WT
50867	CTCTG	A	A
60651	CTCTG	A	A
65722	CTCTG	A	A
66095	CTCTG	A	A
68168	TCTGC	D	D
69174	Negative	WT	WT
69301	Negative	WT	WT
69515	Negative	WT	WT
69674	Negative	WT	WT
69750	Negative	WT	WT
75462	Negative	WT	WT
79236	CTCTG	A	A
84755	Negative	WT	WT
93642	Negative	WT	WT

CONTACT

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For research use (RUO)