

NPM1 MRD Assay

RUO Kit – Now Available for MiSeq™



BACKGROUND

The Nucleophosmin (*NPM1*) gene is one of the most commonly mutated genes in acute myeloid leukemia (AML), occurs in roughly one-third of AML patients at diagnosis^{1,2}, and in combination with other genetic aberrations, may provide information on the course of this disease. The *NPM1* MRD Assay, a targeted, deep sequencing assay, offers a standardized workflow for high-throughput laboratories. Using previously isolated DNA to identify and track mutations 'A', 'B', 'D', and 'Other', the *NPM1* MRD Software reports mutations at an allelic sensitivity of 5×10^{-5} .

To further simplify your workflow and improve throughput, *NPM1* MRD Assay includes 24 unique dual-indices enabling the ability to multiplex multiple samples and targets such as the *FLT3* ITD Assay. This kit configuration provides laboratories the flexibility to scale testing for variable AML MRD research needs.

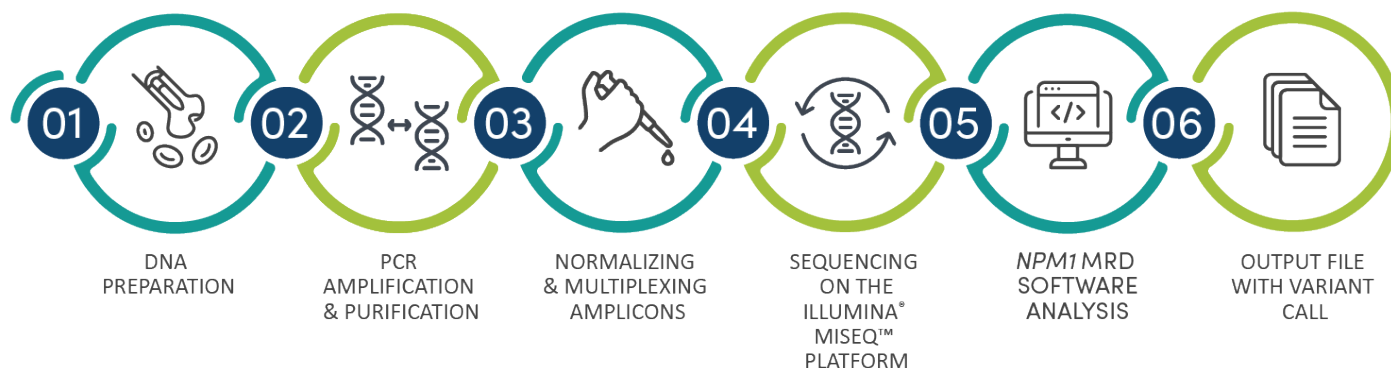
KEY BENEFITS

- » Bring MRD testing in-house for faster turn-around time
- » Allelic sensitivity level of 5×10^{-5}
- » Batch samples using previously isolated gDNA
- » Reduce errors with a standardized workflow
- » Flexibility to multiplex samples and targets to gain cost-efficiencies
- » Dockerized Software automates pipelines for high-throughput labs

PRINCIPLE OF THE PROCEDURE

NPM1 mutations are caused by four-base pair nucleotide insertions most commonly in exon 12 of the *NPM1* gene² on chromosome 5. Next-generation sequencing of the PCR products is used to identify DNA sequences specific to previously identified mutations and estimate variant read frequencies (VRF). The Dockerized *NPM1* MRD Software, provides an objective variant call in a tab separated value (TSV) output file to automate AML MRD studies.

WORKFLOW



ORDERING INFORMATION

Catalog #	Product	Quantity
14160019	<i>NPM1</i> MRD Assay (MiSeq™)	96 reactions
14160029	<i>NPM1</i> MRD Software (MiSeq™)	1 Dockerized Application

REAGENTS INCLUDED IN THE KIT

Controls	Quantity
<i>NPM1</i> Positive Control	500 µL tube x 2 each
<i>NPM1</i> Negative Control	500 µL tube x 2 each
Master Mixes	Quantity
<i>NPM1</i> Master Mixes	75 µL tube x 24 each

Figure 1.0 *NPM1* MRD Software TSV file output example

Our locally-sourced ***NPM1* MRD Software** also provides sequence annotations such as transcript name, coding sequence, amino acid change, chromosomal location, and sequence contig information for every MiSeq™ run in an easily accessible TSV file.

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Output File Example - Notepad
File Edit Format View Help

NPM1 MRD Result:      Positive

Top NPM1 Variant:
TYPE  REFSEQ  CDS    AA      HG19_COORDINATES  READS  VRF    CONTIG_SEQ
Type D  NM_002520.7  c.863_864insCCTG  p.W288Cfs*12  chr5:170837544  491    9.87e-04  GCTATTCAAGATCTCTGCCTGGCAGTGGAGGAAGTCTCTTTAA

Sample Name:  <sample>
Input DNA:    700
Read Depth:   98787

NPM1 MRD Result:      Undetermined

Confidence for the detection of an NPM1 insertion variant with an input VAF of 5.0e-05:
81.52

Minimum NPM1 Insertion Variant VAF detectable with >95% confidence:
7.4e-05

Sample Name:  <sample>
Input DNA:    700
Read Depth:   2245637

NPM1 MRD Result:      Not Detected

Confidence for the detection of an NPM1 insertion variant with an input VAF of 5.0e-05:
100.00

Minimum NPM1 Insertion Variant VAF detectable with >95% confidence:
5.0e-5

Sample Name:  <sample>
Input DNA:    700
Read Depth:   120845

NPM1 MRD Result:      Positive

Top NPM1 Variant:
TYPE  REFSEQ  CDS    AA      HG19_COORDINATES  READS  VRF    CONTIG_SEQ
Type A  NM_002520.7  c.863_864dupTCTG  p.W288Cfs*12  chr5:170837543  9564    7.91e-02  GCTATTCAAGATCTCTGTCTGGCAGTGGAGGAAGTCTCTTTAA

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REFERENCES

- Thiede C, et al. (2006) *Blood* 107:4011-4020.
- Falini et al. (2005) *N Engl J Med* 352:254-66