Instructions for use

NPM1 dPCR OncoKit

Ref. **IMG-432**

RUO

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01 General information

Acute myeloid leukemia (AML) is a hematologic malignancy of the myeloid lineage, characterized by the rapid proliferation of immature cells that accumulate in the bone marrow. Among the various events involved in the pathogenesis of this cancer, mutations of the *NPM1* gene, which codes for the nucleophosmin protein *(NPM1)*, affect around 30% of AML patients, primarily leading to tumors without karyotype abnormalities.

The NPM1 protein, located in the nucleolus, is involved in a number of functions, including regulation of the ARF-p53 tumor suppressor pathway, duplication of centrosomes, and biogenesis and transport of ribosomes, among others. *NPM1* mutations largely take place in exon 12 of the gene with an insertion of 4 nucleotides, which alters the reading pattern. The most common mutation variants are A, B, and D:

```
Wild-type      TATTCAAGATCTCTG----GCAGTGGAGGAAGTC
Variant A      TATTCAAGATCTCTCTCTGGCAGTGGAGGAAGTC
Variant B      TATTCAAGATCTCTGCATGGCAGTGGAGGAAGTC
Variant D      TATTCAAGATCTCTGCCTGGCAGTGGAGGAAGTC
```

In the latest WHO classification, AML disorders with a mutated nucleophosmin (also known as NPMc+) constitute a clinicopathological and molecular entity with clinical, risk stratification, prognostic, and therapeutic implications. As such, WHO has established a simultaneous study of the *FLT3* gene, given that mutations of the *NPM1* gene tend to have a better prognosis when they do not occur alongside other abnormalities, such as FLT3-ITD mutations.

References

- *Heath EM, Chan SM, Minden MD, Murphy T, Shlush LI, Schimmer AD. Biological and clinical consequences of NPM1 mutations in AML. Leukemia. 2017; 31: 798–807.*
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- *Lagunas-Rangel, Francisco Alejandro. "Leucemia mieloide aguda. Una perspectiva de los mecanismos moleculares del cáncer." Gaceta Mexicana de Oncologia 15.3 (2016): 150–157.*

02 Intended use

NPM1 dPCR OncoKit is designed for detecting and quantifying pathogenic variants of the *NPM1* gene based on complementary DNA (cDNA) samples from the peripheral blood of patients diagnosed with or suspected of having AML.

The kit employs a combination of oligonucleotides and fluorescent hydrolysis probes in a multiplex assay for the detection and quantification of A, B, and D pathogenic variants located in exon 12 in comparison to the *wild-type* form of *NPM1*, using digital PCR (dPCR) technology.

NPM1 dPCR OncoKit is designed exclusively for research use and is strictly intended for technical healthcare and molecular biology professionals who need to identify and quantify *NPM1* mutations.

IMPORTANT: The kit is not designed to determine suspension of the treatment or to monitor a patient after suspension of the treatment.

03 Technical characteristics

NPM1 dPCR OncoKit is designed for analyzing DNAc extracted from peripheral blood.

For validation of NPM1 dPCR OncoKit, a DNA probe was designed with the A, B, and D variants, along with a region of the *wild-type NPM1* gene. The detection and quantification of different alleles enables us to calculate the frequency of the variant of interest *(VAF, variant allele frequency)* in the sample. We also analyzed samples from patients previously genotyped with other forms of technology.

Comprehensive validation allows to establish the following **technical specifications**:

- **Type of sample**: cDNA from peripheral blood
- **Positive control**: DNA probe with the pathogenic variants (A, B, and D) and a *wild-type NPM1* region.
- **Limit of detection**: 0.04% ± 0.012
- **Limit of quantification**: 0.1%
- **Number of reactions per sample**: 1
- **Number of targets**: 4 (A, B, and D variants in *NPM1* and a *wild-type NPM1* region serving as an endogen)
- **dPCR program duration**: 01:45.

NPM1 dPCR OncoKit is compatible with the *QuantStudio™ Absolute Q™ Digital PCR System* platform (Thermo Fisher Scientific) with FAM™ and VIC™ fluorescence channels. The device uses *QuantStudio Absolute Q Digital PCR System Software* for semi-automated result analysis.

04 Conditions for sample collection, handling and preparation

Sample collection, transport, storage, and preparation are critical steps for performing assays for mRNA transcript detection and quantification. Some of the most important requirements for the handling of these samples are highlighted below. The stability of the samples under transport and storage conditions other than those listed below has not been evaluated with NPM1 dPCR OncoKit.

- Sample collection and storage.

  For optimal results, whole blood samples should be collected in *PAXgene* tubes intended for intracellular RNA purification from whole blood, following the manufacturer's guidelines. If RNA extraction is not performed immediately after collection, it is recommended to store the samples at 4°C for up to 5 days without separating the plasma from the cells. For longer storage, it is recommended to freeze the specimens at -20°C to -70°C.

- Sample transport:

  If mRNA extraction is performed in a laboratory other than the one where the sample was collected, it is recommended to ship the sample within 5 days of collection, at 4°C and on dry ice.

- RNA extraction, working conditions and storage:

  Use a suitable manual or automated human RNA extraction method. The yield and purity of extracted RNA may differ depending on the extraction method. The *Maxwell® 16 LEV simplyRNA Tissue Kit* (Promega, ref: AS1280) with the corresponding *Maxwell® 16 Instrument* (Promega) has been successfully used for automated extraction.

  Work surfaces and equipment should be thoroughly cleaned with a special RNAse decontamination solution prior to RNA extraction, and all consumables should be RNase-free.

  mRNA should be kept at a temperature of 4°C for immediate use. For short-term and long-term use, it is recommended to keep samples frozen at -20°C and -80°C, respectively. Repeated freezing and thawing of RNA is discouraged as it may affect its integrity.

- Reverse transcription (RT-PCR) and storage of cDNA:

  Reverse transcription of mRNA to cDNA was performed with the *SuperScript™*

VILO™ cDNA Synthesis Kit (Thermo Fisher Scientific) using two-step RT-PCR and 1,000 ng mRNA. Using lower amounts of starting material may adversely affect the detection of *NPM1* transcripts that are underrepresented in the patient's blood.

The cRNA should be kept at a temperature of 4°C for immediate use. For short-term and long-term use, it is recommended to keep samples frozen at -20°C and -80°C, respectively.

05 Warnings and precautions

- We recommend strictly following the instructions in this manual, especially regarding the handling and storage conditions for the reagents.
- RNAase/DNase contamination can lead to the degradation of RNA samples and cDNA products. Use pipette tips with filters and nuclease-free tubes. Thoroughly clean all work surfaces before commencing work.
- The diagnostic efficacy of this test has only been established for blood collected in *PAXgene* tubes. The performance of this test has not been evaluated with other specimen types or collection methods.
- The reliability of the results depends on the correct performance of specimen collection, transport, storage, and processing as outlined in section "04. Conditions for sample collection, handling, and preparation". Other sample handling issues may lead to inaccurate results.
- Samples with target transcript concentrations below the established limit of detection may lead to erroneous results.
- Risk prevention and good laboratory practice.
- Any skin conditions, cuts, abrasions, and other skin lesions should be properly covered or protected.
- Do not pour reagent residues into the drinking water system. It is recommended to use the waste containers set out by the legal regulations and to manage them via an authorized waste manager.
- In the case of accidental spillage of any of the reagents, avoid contact with skin, eyes, and mucous membranes and wash with plenty of water.
- Product Material Safety Data Sheets (MSDS) are available at https://healthincode.com/kits-de-analisis/.
- This product requires the handling of samples and materials of human origin. It is recommended to consider all materials of human origin as potentially infectious and to handle them in accordance with the OSHA Biosafety Level 2 standard for bloodborne pathogens. Alternatively, other relevant biosafety practices should be used for materials that contain or may contain infectious agents.
- The reagents included in this kit are not toxic, explosive, infectious, radioactive, magnetic, or corrosive and do not cause biological environmental contamination.

- This kit has been validated with specific equipment and under specific conditions that may vary significantly in other laboratories. It is therefore recommended that each laboratory perform an internal validation when using the kit for the first time.
- The manufacturer is not responsible for the assay failing to work properly when the reagents included in the kit are replaced by other reagents not supplied by Health in Code, S.L.
- The manufacturer does not guarantee the reproducibility of the assay when the user includes reagents not validated by Health in Code S.L., considering them equivalent to those supplied in the kit.

06 Kit contents and storage conditions

NPM1 dPCR OncoKit is composed of the following reagents:

- *NPM1 Master Mix*, which contains:
  - specific oligonucleotides.
  - probes made by Applied Biosystems™ labeled with FAM™ and VIC™ for detecting the A, B, or D variants of *NPM1* and the *wild-type* form of the *NPM1* gene, respectively.
  - nuclease-free water.
- Positive control: Contains a DNA probe with the A, B, and C variants and the *wild-type* form of *NPM1*.

The kit contains enough lyophilized reagents for 24 digital PCR reactions. Physical characteristics, quantities, and storage conditions are shown in Table 1.

Table 1. Components of NPM1 dPCR OncoKit.

Reagents	Color of the carrier	Quantity	Storage
NPM1 Master Mix	Yellow pad	2 X 12 rxn	4°C
Positive control	Yellow cap	6 rxn	4°C

07 Equipment, reagents and materials not supplied

Equipment:

- 10 µL, 20 µL, and 200 µL micropipettes
- Vortex
- Centrifuge
- *QuantStudio Absolute Q Digital PCR* (Thermo Fisher Scientific)

Reagents:

- Nuclease-free water
- Absolute ethanol
- *Absolute Q DNA Digital PCR Master Mix (5X)* (Ref. A52490)
- *Isolation Buffer* (Ref. A52730)
- *SuperScript™ VILO™ cDNA Synthesis Kit* (Thermo Fisher Scientific)

Materials:

- Pipette tips with filter (10 µL, 20 µL, and 200 µL)
- 1.5 mL sterile tubes
- Powder-free latex gloves
- *MAP16 Plate Kit* (Ref. A52865)

Complementary kits

For the investigation of hematological disorders, Health in Code, S.L. also offers the following real-time PCR kits for specific targets:

- Imegen® BCR ABL1 Screening (ref. IMG-108)
- Imegen® m-BCR ABL (ref. IMG-121)
- Imegen® M-BCR ABL (ref. IMG-122)
- Imegen® Inv16 (ref. IMG-109)
- Imegen® PML-RARA Screening (ref. IMG-130)
- Imegen® PML-RARA (ref. IMG-111)
- Imegen® NPM1 (ref. IMG-235)
- Imegen® MPL (ref. IMG-236)

By fragment analysis:

- Imegen® FLT3 (ref. IMG-238)
- Imegen® CALR (ref. IMG-237)

By digital PCR (dPCR) analysis:

- BCR-ABL1 Screening dPCR OncoKit (ref. IMG-424)
- FLT3-TKD2 dPCR OncoKit (ref. IMG-405)
- cKIT dPCR OncoKit (ref. IMG-407)
- mBCR-ABL dPCR Oncokit (ref. IMG-429)
- RUNX1-RUNX1T1 dPCR Oncokit (ref. IMG-430)
- ETV6-RUNX1 dPCR Oncokit (ref. IMG-431)
- MYD88 dPCR Oncokit (ref. IMG-434)

By Next-Generation Sequencing (NGS):

- Haematology OncoKitDx (ref. IMG-363)

08 Assay protocol

08.1 | Preparation of PCR digital reagents

The first step before starting the protocol is to rehydrate the dried reagents:

- *NPM1 Master Mix*: Add 20 µL of nuclease-free water to each vial.
- Positive control: Add 50 µL of nuclease-free water to the vial.

NOTE: It is recommended to store the rehydrated reagents frozen at – 20°C.

08.2 | Preparation of amplification reactions

In order to estimate the quantity of reagents required, the number of samples and controls to be analyzed simultaneously must be taken into account. We recommend adding one more reaction or increasing the volume of each reagent by 10% when making the calculations (item 4 of the protocol).

In each assay, it is recommended to include the positive control provided in the kit and a negative PCR control to rule out contamination of the reagents.

The protocol for the preparation of amplification reactions is specified below:

01 Thaw the cDNA samples.

NOTE:

> Take into account that prior dilution of the cDNA may be required, as indicated in section "10. Troubleshooting".

> Samples of suboptimal quality should be centrifuged at 10,000 x g or at the maximum available speed for 1 minute; the sample should then be drawn from the top of the tube.

02 Thaw all the reagents in the kit, the nuclease-free water and the *Absolute Q DNA Digital PCR Master Mix* (5X) (not included in the kit). It is important to allow all the components to reach room temperature before use.

NOTE: Keep all the reagents on ice and protected from light when not in use.

03 Vortex all the reagents, including *Absolute Q DNA Digital PCR Master Mix* (5X), for 10 seconds.

04 Prepare the PCR mix in a 1.5 mL tube with the reagents listed below.

Reagent	Volume per reaction**
NPM1 Master Mix	1.5 µL
*Absolute Q DNA Digital PCR Master Mix (5x)**	2 µL

(*) Reagent not supplied by the manufacturer (see section "07. Equipment, reagents, and materials not supplied").

(**) Adjust the final volume according to the number of reactions as described at the beginning of the section.

05 Vortex the PCR mix for 10 seconds and then spin for 30 seconds. Aliquot 3.5 µL into different PCR tubes.

06 Transfer 6.5 µL of the supernatant from the samples into each PCR tube containing the reaction mix prepared in step 4. For the negative control, add nuclease-free water.

07 Use of the following options to add the mix from item 6:

- Pipette up and down 10–20 times, taking care not to create bubbles.
- Vortex 3–5 times for 1 second at a time.

08 Centrifuge for 1 min at 10,000 x g.

09 Hold the pipette at a 45° angle and load 9 µL of the PCR mix into the bottom of each well of the MAP16 microfluidic array plate, depressing until the first pipette stop.

10 Then, holding the pipette tip against the side of the well at a 45° angle, dispense 15 µL of Isolation Buffer on top of the reagents to prevent mixing and bubble formation.

Optional: Wait 15 min before pressing Start and keep the plate inside the device in case bubbles have formed or the reagents have mixed during loading.

08.3 | Real-time PCR program setup

Load the microfluidic array plate (MAP16)

Mount the strips for the MAP17 plates in the 4 well columns. Make sure the columns are completely covered and place the MAP plate caps on all the columns, including unused ones. Download the user guide: *MAN0025621 QuantStudio™ Absolute Q™ Digital PCR System User Guide* from www.thermofisher.com, and follow the instructions provided in Section 2.

PCR program setup

Place the MAP plate in the *QuantStudio™ Absolute Q™ System*, adjust the optimal channels and PCR settings as specified in Table 2 and set up the dPCR program as described in Table 3.

- Experiment setup:

Table 2. Channel setting for fluorophore detection on the QuantStudio™ Absolute Q™ dPCR System. QC: Quality control.

Fluorophore	Molecular target	Analysis
FAM™	A, B, and D variants of NPM1	Signal
VIC™	Wild-type NPM1	Signal
ROX™	QC	QC

- Optimal PCR program:

Table 3. Optimal PCR program for QuantStudio™ Absolute Q™ dPCR (Thermo Fisher Scientific).

Stage	No. of cycles	Temperature	Time
Initial denaturation	1	96°C	10 minutes
PCR Denaturation, annealing, and extension	40	96°C	5 seconds
		62°C	15 seconds

↘ Array reading and obtaining results

Following completion of the PCR, follow the instructions in the user guide for the *QuantStudio™ Absolute Q™* digital PCR system available from www.thermofisher.com.

09 Analysis of results

The approach used in the design of NPM1 dPCR OncoKit is based on a competitive multiplex dPCR assay in which the A, B, and D variants of *NPM1* are detected in comparison to the *wild type* of the gene, as shown in Table 4.

Table 4. Information on the targets for each probe used in NPM1 dPCR OncoKit

Fluorophore	Function
FAM™	The FAM™ probe identifies the A, B, and D variants of *NPM1*
VIC™	The VIC™ probe identifies the *wild-type NPM1* gene

Sample analysis is accomplished by the specific software of the PCR thermocycler used: *QuantStudio™ Absolute Q™ Digital PCR Software*. To determine whether a sample is positive and to quantify it, follow the steps below.

01 Go to the *"ANALYSIS"* section to visualize and select a sample.

02 At the bottom of the screen, choose the channel you want to display (FAM™ for the A, B, and D variants of *NPM1* and VIC™ for the *wild-type NPM1* gene).

03 Select option  to display the data in a two-dimensional (2D) or scatter plot. After adjusting the X and Y axes in the 2D plots, up to four clusters may be identified (Figure 1):

- The cluster of black data points represents the sections where there has been no amplification and, therefore, no signal has been recorded for any of the probes.
- The cluster of orange data points corresponds to positive areas for the VIC™ probe, which hybridizes with a region of the *wild-type NPM1* gene serving as the endogenous control.
- The cluster of purple data points corresponds to positive areas for the FAM™ probe, which hybridizes with the A, B, and D variants of *NPM1*.
- The cluster of green data points, or combined group, corresponds to positive areas where signals were detected for both variants of the *NPM1* gene and the *wild-type NPM1* gene.

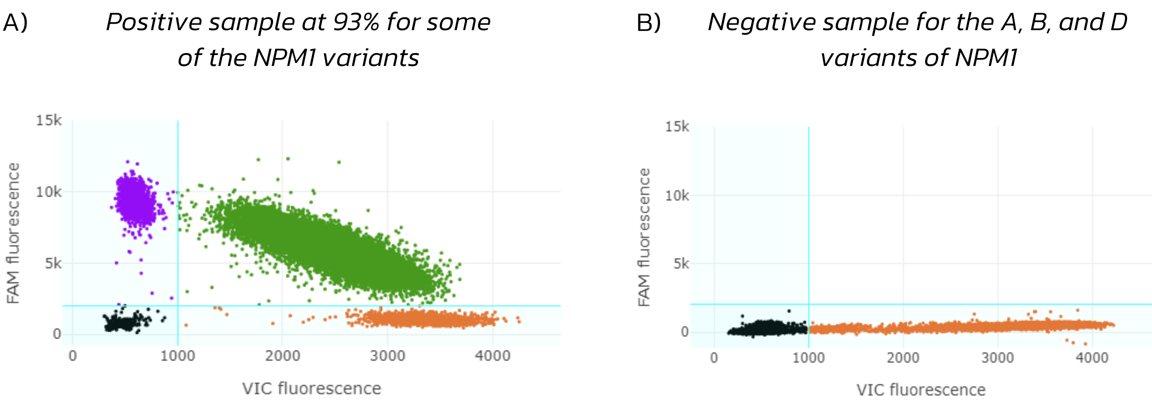

Figure 1. Two-dimensional (2D) plots for quantification of the A, B, and D variants of NPM1 in comparison to the wild-type NPM1 with the NPM1 dPCR OncoKit assay. ***A)*** *Positive sample at 93% for some of the NPM1 variants. The observed plot is obtained by selecting the specific fluorescence channel for the transcript (FAM™) relative to VIC (VIC™).* ***B)*** *Negative sample for variants in NPM1. No specific amplification was observed for the A, B, or D variants. In all cases, the cluster of black data points represents areas where no amplification has occurred.*

Table 5 summarizes the interpretation of possible results for NPM1 dPCR OncoKit.

*Table 5. Possible outcomes when loading a cDNA sample, with + and – indicating the presence or absence of clusters. *The combined channels correspond to the green clusters, where the transcripts corresponding to the NPM1 variants are amplified along with the wild-type NPM1.*

Interpretation of results			
Sample	Channel FAM™	Channel VIC™	Combined channels *
Positive for the A, B, or D variants of *NPM1*	+	+	+
Wild-type NPM1 sample	–	–	–

Calculation of the frequency of the A, B, or D variants of *NPM1* in the sample

The analytical software analyzes the graphs obtained and reports the results in copies per microliter (copies/µL) based on the concentration of the targets in the dPCR reaction. This data is used to quantify the transcripts of the *NPM1* variants expressed as a relative measure in relation to the *wild-type* gene, according to the following formula:

$$\%\, NPM1m = \frac{NPM1m\ cp/\mu l\ \ (FAM)}{NPM1m\ \frac{cp}{\mu L}\ (FAM) + NPM1wt\ \frac{cp}{\mu L}(VIC)}\ x\ 100$$

Where:

- **NPM1m (mutated *NPM1*)**: Represents the copies/µL in FAM™ reported by the analysis program.
- **NPM1wt (*wild-type NPM1*)**: Represents the copies/µL for VIC™ reported by the analysis program.

For the assay to be considered valid, the user must ensure that the following conditions are met:

- There is no amplification signal from the PCR negative control in any of the configured channels.
- There is a signal in all channels (FAM™ and VIC™) in the reaction with the positive control.
- If other results than those shown in this section are obtained, please refer to section "10. Troubleshooting" of this manual.

10 Troubleshooting

Table 6 presents the expected results of an NPM1 dPCR OncoKit assay for controls and samples, as well as INVALID combinations, their potential causes, and solutions.

Table 6. Possible outcomes of the assay. Includes invalid results, their possible causes, and solutions

Control	Result		Cause
	FAM™	VIC™	
Positive control	+	+	Expected result
	–	–	Failed PCR amplification[1]
Sample	–	+	Expected result
	+	+	
	+	+	Overlapping clusters[2]
	+	–	Invalid result[3]
	–	–	Failed PCR amplification[1] Sample amplification error[4]
PCR Negative control	–	–	Expected result
	+	+	Contamination of PCR with human DNA[5]
	+	–	
	–	+	

(1) Failed PCR amplification: Check the amplification program and fluorescence capture settings. Amplification failures may result from technical issues related to PCR program settings or errors in preparing the reaction mixes.

(2) Saturation due to cDNA overload: Samples with high concentrations of NPM1m or NPM1wt transcripts may generate signals in the corresponding channels, but with significant noise. In such cases, it is recommended to dilute the sample.

(3) Invalid result: No amplification of VIC™ (*ABL1* endogenous control). It is recommended to repeat the test.

(4) Sample amplification failure: The quality of the sample directly affects PCR performance. If the sample is highly degraded, lack of amplification may occur. On the other hand, if RT-PCR is performed with higher amounts of RNA than recommended, amplification failure may be due to saturation from excess cDNA. In such cases it is recommended to dilute the sample 1/10. If the clusters have not been distributed satisfactorily as shown in Figure 1 of section "9. Results analysis", readjust until you obtain concentrations of 1500–2500 copies/µL, according to the concentration obtained.

(5) Contamination of the PCR process with human DNA: this may result from mishandling of the sample, use of contaminated reagents or environmental contamination. Thoroughly clean the laboratory where the PCR was prepared, as well as the equipment and materials used. If necessary, use new aliquots of PCR reagents. Prepare the PCR reaction containing the positive control as the final step, in order to avoid cross-contamination. In this case, it is recommended to repeat the test.

11 Limitations

11.1 | Tests

NPM1 dPCR OncoKit is designed to detect and quantify the A, B, and D variants of *NPM1* mutations. We have not assessed the capacity to detect other mutation variants.

Lower mRNA starting quantities than indicated during reverse transcription (see section "04. Conditions for sample collection, handling, and preparation") may adversely affect the detection of *NPM1* variants present in very low numbers in the patient's blood.

In samples with a high concentration of *NPM1*, NPM1 dPCR OncoKit may generate INVALID results due to system saturation. In such cases, it is recommended to repeat the test with a diluted sample. For more information, see Table 6, section "10. Troubleshooting".

The assay may detect certain *NPM1* variants in patients even at very low levels (below the LoD of 0.045%). However, its accuracy is not guaranteed below the stated LoD.

11.2 | Equipment

NPM1 dPCR OncoKit has been validated using the following amplification platform:

- *QuantStudio™ Absolute Q™ Digital PCR System* (Thermo Fisher Scientific)

11.3 | Reagents

NPM1 dPCR OncoKit has been validated using the reagents included in the kit and those recommended in section 7 of this manual (Equipment, reagents, and materials required but not supplied).

This kit is validated with blood collected in *PAXgene* tubes. Modifications to these procedures may affect test performance.

This product is validated with the *SuperScript™ VILO™ cDNA Synthesis Kit* (Thermo Fisher Scientific). Modifications to these procedures may affect test performance.

11.4 | Product stability

The optimum performance of this product is confirmed provided that the recommended storage conditions according to the optimum product date for each production batch are followed.

12 Performance characteristics

12.1 | Validation samples

NPM1 dPCR OncoKit is designed for analyzing the total complementary DNA (cDNA) extracted from peripheral blood samples.

For the analytical validation of NPM1 dPCR OncoKit, a DNA probe was designed that contains the A, B, and D variants of *NPM1*, using a *wild-type NPM1* region as an endogenous control.

The kit was also validated with 15 positive clinical samples and 15 negative samples to determine the sensitivity and specificity of the kit.

12.2 Limit of Blank (LoB) and Limit of Detection:(LoD)

The LoB and LoD of NPM1 dPCR OncoKit were determined using the *QuantStudio™ Absolute Q™ Digital PCR System*.

Fifteen negative samples were used to calculate the LoB. The expected negative result was obtained in all cases, making it possible to establish an LoB with a 95% confidence interval (CI) of 0.003%. Taking into account the LoB obtained, the LoD was evaluated on 4 replicates of the corresponding DNA probe mixes to achieve an allele frequency of 0.01% from each of the *NPM1* variants (A, B, and D). The results enabled us to establish an LoD at 0.04% ± 0.012 with a CI of 95%.

12.3 | Limit of quantification (LoQ)

The limit of quantification (LoQ) for NPM1 dPCR OncoKit was estimated using 4 replicates with DNA probes diluted to an allele frequency of 0.1%. The results obtained meet the acceptance criteria to establish the LoQ at 0.1% (CV <25%).

12.4 | Analytical specificity

The analytical specificity of NPM1 dPCR OncoKit was determined by analyzing 15 negative samples for variants in *NPM1*. We did not detect a signal above the established LoD in any of the samples, so were able to confirm 100% specificity for the system.

12.5 | Reproducibility and repeatability

The repeatability and reproducibility performance parameters of NPM1 dPCR OncoKit were evaluated using DNA probes with the *NPM1* variants of interest at the established LoQ (0.1%).

To determine the repeatability of the assay, we loaded 4 replicates of a DNA probe mixture at 10%, 1%, and 0.1% for each of the variants, obtaining the following quantifications:

- **Variant A 10%**: 10.50 ± 0.25 (SD) and a variation coefficient of 2.7%.
- **Variant A 1%**: 1.18 ± 0.03 (SD) and a variation coefficient of 2.6%.
- **Variant A 0.1%**: 0.14 ± 0.20 (SD) and a variation coefficient of 14.3%.

- **Variant B 10%**: 11.01 ± 0.12 (SD) and a variation coefficient of 1.2%.
- **Variant B 1%**: 1.21 ± 0.08 (SD) and a variation coefficient of 6.4%.
- **Variant B 0.1%**: 0.14 ± 0.03 (SD) and a variation coefficient of 20.8%.

- **Variant D 10%**: 11.16 ± 0.11 (SD) and a variation coefficient of 1.0%.
- **Variant D 1%**: 1.29 ± 0.09 (SD) and a variation coefficient of 6.7%.
- **Variant D 0.1%**: 0.14 ± 0.015 (SD) and a variation coefficient of 10.5%

For reproducibility, one replicate per assay was analyzed on three different days in the following range of concentrations: 0.1%, 1%, and 10% for each of the *NPM1* variants. The coefficient of variation never exceeded 25%, demonstrating excellent accuracy for the NPM1 dPCR OncoKit assay.

12.6 | Analytical sensitivity

To determine the analytical sensitivity of NPM1 dPCR OncoKit, 15 positive clinical samples were analyzed for the *NPM1* variants assessed with qPCR in the hospitals of origin. The NPM1 dPCR OncoKit assay identified all the samples as positive, thus establishing a sensitivity of 100%.

Contact our Technical Department for any questions about the applications of this product or its protocols:

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