July 2023

NeuMoDx™ LDT Master Mix, DNA Instructions for Use

Σ 96

Version 1

IVD

For In Vitro Diagnostic Use with the NeuMoDx 288 and NeuMoDx 96 Molecular Systems

℞ only

For prescription use only

CE

REF 210100

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NeuMoDx™ molecular
a QIAGEN company

For detailed instructions, refer to the *NeuMoDx 288 Molecular System Operator's Manual*; P/N 40600108

For detailed instructions, refer to the *NeuMoDx 96 Molecular System Operator's Manual;* P/N 40600317

Contents

Intended Use

NeuMoDx LDT Master Mix, DNA is a 16-well strip containing a proprietary, room-temperature stable, real-time PCR master mix that, when used in conjunction with assay specific primers and probe(s), enables a laboratory to rapidly develop and implement laboratory developed tests (LDTs) on the NeuMoDx 288 Molecular System and NeuMoDx 96 Molecular System (NeuMoDx System(s)). Other than the LDT-specific primers and probe(s), the NeuMoDx LDT Master Mix, DNA incorporates all the reagents required for real-time PCR. Once validated by the user's laboratory as part of the LDT, this reagent can be used as a key component for rapid automation of the LDT.

Summary and Explanation

Laboratory developed tests incorporating the NeuMoDx LDT Master Mix, DNA and implemented on the NeuMoDx System offer clinical laboratories a simple, efficient, and straightforward way to rapidly integrate LDTs for sample-to-result operation. The NeuMoDx System incorporates extraction, purification, amplification, and results interpretation. The System allows for the combination of its universal nucleic acid isolation process with the use of the NeuMoDx LDT Master Mix, DNA and general purpose real-time PCR reagents to deliver highly accurate results for LDTs from unprocessed clinical samples. The user simply provides assay-specific primers and probe(s) in a separate NeuMoDx LDT Primer/Probe Strip [REF 100400] and defines the desired real-time PCR thermal profile. Once the clinical specimens and assay specific reagents are properly loaded on the NeuMoDx System, the System automatically starts processing the samples.

Principles of the Procedure

The NeuMoDx Systems use a combination of heat, lytic enzymes, and extraction reagents to perform cell lysis, DNA extraction, and inactivation/removal of inhibitors from unprocessed clinical specimens prior to presenting the extracted DNA for detection by real-time PCR. Upon lysis, the released nucleic acids are captured by paramagnetic particles. The particles, with the bound nucleic acids, are then loaded into the NeuMoDx Cartridge where the unbound/non-specifically bound components are washed away using the NeuMoDx Wash Reagent and the bound DNA is eluted using NeuMoDx Release Reagent. The NeuMoDx System mixes the released DNA with the user provided LDT primers and probe(s) then uses an aliquot of this solution to rehydrate the dried assay reagents in the NeuMoDx LDT Master Mix, DNA, which contains all the reagents necessary to perform real-time PCR: Taq DNA polymerase, dNTPs, $MgCl_2$, and other optimized excipients and buffering agents. These dried assay reagents also contain the components required to amplify a section of the sample process control (SPC1) sequence, enabling simultaneous amplification and detection of both target and internal control DNA sequences. The dried assay reagents in the NeuMoDx LDT Master Mix, DNA do not contain any LDT-specific primers or probes (assay specific reagents) other than the SPC1 primers and probe; the assay specific reagents must be added by the user to the NeuMoDx LDT Primer/Probe Strip. Upon mixing with the user-provided primers and probe(s) and reconstitution of the dried PCR reagents, the NeuMoDx System dispenses the prepared PCR-ready mixture into the NeuMoDx Cartridge. Amplification and detection of the control and target (if present) DNA sequences occur in the PCR chamber of the Cartridge. The chamber and the Cartridge are designed to contain the amplicon following real-time PCR and essentially eliminate contamination risk post amplification.

Once the PCR chamber is loaded by the NeuMoDx System with the reagents, real-time PCR occurs. The amplified targets are detected in real time using hydrolysis probe chemistry (commonly referred to as TaqMan® chemistry) using fluorogenic oligonucleotide probe molecules specific to the amplicons for their respective targets. TaqMan probes consist of a

fluorophore covalently attached to the 5'-end of the oligonucleotide probe and a quencher at the 3'-end. While the probe is intact, the fluorophore and the quencher are in proximity, resulting in the quencher molecule extinguishing the fluorescence emitted by the fluorophore via FRET (Förster Resonance Energy Transfer).

TaqMan probes are designed to anneal within a target region amplified by a specific set of primers. As the Taq polymerase extends the primer and synthesizes the new strand, the 5' to 3' exonuclease activity of the Taq polymerase degrades the probe that has annealed to the template. Degradation of the probe releases the fluorophore and breaks its proximity to the quencher, thereby overcoming the quenching effect due to FRET and allowing fluorescence of the fluorophore. The resulting fluorescence signal detected in the quantitative PCR thermal cycler is directly proportional to the fluorophore released and can be correlated to the amount of target DNA present.

For detection of the Sample Process Control, the TaqMan probe is labeled with a fluorescent dye (535/556 nm) at the 5' end, and a dark quencher at the 3' end. The NeuMoDx System monitors the fluorescent signal emitted by the TaqMan probes at the end of each amplification cycle. When amplification is complete, the NeuMoDx System software presents the amplification curves of each sample for analysis by end user.

Materials Provided

Kit contents

NeuMoDx LDT Master Mix, DNA REF 210100	**Units per Package**	**Tests per Unit**	**Tests per Package**
NeuMoDx LDT Master Mix, DNA *Dried RT-PCR reagents containing Sample Process Control 1 specific TaqMan probe and primers.*	6	16	96

Materials Required but Not Provided

REF	Contents
100100	**NeuMoDx Cartridge**
100200	**NeuMoDx Extraction Plate** ***Dried paramagnetic particles, lytic enzymes, and sample process controls***
various	**NeuMoDx Lysis Buffer(s)**
400100	**NeuMoDx Wash Reagent**
400200	**NeuMoDx Release Reagent**
100400	**NeuMoDx LDT Primer/Probe Strip**
235903	**Hamilton CO-RE / CO-RE II Tips (300 µl) with Filters**
235905	**Hamilton CO-RE / CO-RE II Tips (1000 µl) with Filters**

Reagents

- 10 mM Tris-HCl pH 8.0, RNase/DNase Free Water, or TE Low EDTA (0.1 mM)
- LDT primers and probe(s)

Equipment*

- NeuMoDx 288 Molecular System [REF 500100] OR
  NeuMoDx 96 Molecular System [REF 500200]

* Prior to use, ensure that instruments have been checked and calibrated according to the manufacturer's recommendations.

Warnings and Precautions

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/neumodx-ifu**, where you can find, view and print the SDS for each NeuMoDx kit and kit component.

- For *in vitro* diagnostic use with NeuMoDx Systems only.
- Do not use the reagents after the listed expiration date.
- Do not use if the packaging is damaged or if foil pouch is open or broken upon arrival.
- Do not reuse any NeuMoDx consumable or reagent.
- Minimum specimen volume is dependent on the aspirate volume and tube size. See the NeuMoDx System Operator's Manuals and LDT Supplement for details. Volume below the specified minimum may result in a "Quantity Not Sufficient" error.
- Avoid microbial and deoxyribonuclease (DNase) contamination of all reagents and consumables. The use of sterile RNase/DNase-free disposable transferring pipettes is recommended. Use a new pipette for each specimen.
- The use of sterile RNase/DNase-free, filtered disposable pipette tips is recommended for dispensing LDT reagents. Use a new tip for each set of primers and probe(s).
- To avoid contamination, do not handle or break apart a NeuMoDx Cartridge post-amplification. Do not retrieve NeuMoDx Cartridges from the Biohazard Waste Container (NeuMoDx 288 Molecular System) or Biohazard Waste Bin (NeuMoDx 96 Molecular System) under any circumstance. The NeuMoDx Cartridge is designed to prevent contamination.

- In cases where open-tube PCR tests are also conducted by the laboratory, care must be taken to ensure that the NeuMoDx LDT Master Mix, DNA, the additional consumables and reagents required for testing, personal protective equipment such as gloves and lab coats, and the NeuMoDx System are not contaminated.
- Clean, powder-free, nitrile gloves should be worn when handling NeuMoDx reagents and consumables. Care should be taken not to touch the top surface of a NeuMoDx Cartridge, the foil seal surface of a NeuMoDx LDT Master Mix, DNA or NeuMoDx Extraction Plate, or the top surface of a NeuMoDx Lysis Buffer; handling of the products should be done by touching side surfaces only.
- Safety Data Sheets (SDS) are provided for each reagent (as applicable) at www.qiagen.com/neumodx-ifu.
- Always wear clean powder free nitrile gloves when handling specimens or any NeuMoDx reagents or consumables.
- Wash hands thoroughly after performing the test.
- Do not pipette by mouth. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*[1] and in CLSI Document M29-A4.[2]
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.

Precautions

Contains: boric acid. Danger! Causes serious eye irritation. May damage fertility or the unborn child. Obtain special instructions before use. Do not handle until all safety precautions have been read and understood. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF exposed or concerned: Get medical advice/ attention. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

Emergency information

CHEMTREC
Outside USA & Canada +1 703-527-3887

Disposal

Dispose of as hazardous waste in compliance with local and national regulations. This also applies to unused products.

Follow recommendations in the Safety Data Sheet (SDS).

Product Storage, Handling, and Stability

- NeuMoDx LDT Master Mix, DNA is stable in the primary packaging at 15 to 28 °C through the stated expiration date on the immediate product label.
- Do not use reagents past the stated expiration date.
- Do not use if product or packaging has been visually compromised.
- Once loaded, the NeuMoDx LDT Master Mix, DNA may remain onboard the NeuMoDx System for 62 days. Remaining shelf life of loaded Master Mix is tracked by the software and reported to the user in real time. Removal of a Master Mix that has been in use beyond its allowable period will be prompted by the System.
- Onboard stability of LDT primers and probe(s) dispensed into the NeuMoDx LDT Primer/Probe Strip requires validation by the user's laboratory.

Specimen Collection, Transport, and Storage

Handle all specimens as if they are capable of transmitting infectious agents. Validation of optimal specimen shipping conditions and specimen stability should be conducted by the user's laboratory for the sample matrix used and for each type of test performed.

Instructions for Use

Sample Preparation

1. Apply a specimen barcode label to the desired specimen tube. Testing can be run on an aliquot in a secondary tube or directly from the primary specimen tube, if appropriate for assay and compatible with the NeuMoDx System. For additional details, see the *NeuMoDx Operator's Manuals and LDT Supplement*.
2. Ensuring that all caps have been removed from the specimen tubes, load the barcoded specimen tubes into the appropriate Specimen Tube Carrier of the NeuMoDx System.

Test Definition

1. Open the Test Editor Wizard in the NeuMoDx System software under Test Tab in Tools Menu.
2. Follow touchscreen instructions to input all assay specific information.

NeuMoDx System Operation

1. Populate system carriers as necessary with the following consumables and use the touchscreen to load carrier(s) into the NeuMoDx System:
   1a. 1000 µl CO-RE / CO-RE II Tips
   1b. 300 µl CO-RE / CO-RE II Tips
   1c. NeuMoDx Cartridge
   1d. NeuMoDx Extraction Plate
   1e. NeuMoDx LDT Master Mix, DNA
   1f. Relevant NeuMoDx Lysis Buffer
   NOTE: *remove foil seal from containers prior to loading*
2. Replace Wash Reagent and Release Reagent and empty the Priming Waste Bottle, as necessary.

3. Empty Biohazardous Waste as necessary, changing gloves before moving on to the next step.
4. Prepare the LDT primer/probe mix:
   4a. Dilute primers and probe(s) in water, 10 mM Tris pH 8.0, or 1X TE with low EDTA (0.1 mM EDTA). The final concentration of the primer/probe mix should be 1X after mixing with 18 µl of eluate in the NeuMoDx LDT Primer/Probe Strip.
   Example*:* Add 4 µl of 6X Primer/Probe Mix to a well. Once eluate is added to the well and mixed with the LDT Primer/Probe Mix, there will be 24 µl at 1X Primer/Probe Mix.
   4b. NeuMoDx recommends adding between 3 µl and 10 µl of the prepared primer/probe mix per well of the NeuMoDx LDT Primer/Probe Strip.
5. Using a clean pipette tip, pierce the foil on the NeuMoDx LDT Primer/Probe Strip for as many wells as are necessary for the number of tests to be run.
6. Carefully dispense the LDT primer/probe mix into the bottom of the wells to be used on the NeuMoDx LDT Primer/Probe Strip. There is no need to fill all wells, but loading must start from the bottom left well (see figure below). Place the NeuMoDx LDT Primer/Probe Strip in a Test Strip Carrier. Alternatively, snap the strip into place on the Carrier and then load with LDT primer/probe mix.

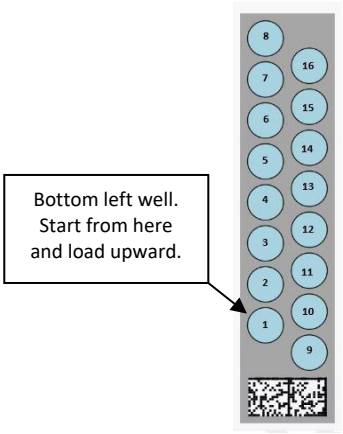

Figure 1. Order for filling LDT primer/probe mix wells

7. Touch the arrow below the desired Test Strip Carrier on the touchscreen to load the NeuMoDx LDT Primer/Probe Strip into the System. The wells will display as yellow. Touch the wells to define the assay type and map the locations on the NeuMoDx LDT Primer/Probe Strip that contain the LDT primer/probe mix.
8. Insert the specimen tube(s) into the appropriate Specimen Tube Carrier, and ensure caps are removed from all specimen tubes.
9. Place Specimen Tube Carrier on the Autoloader shelf and use the touchscreen to load carrier into the NeuMoDx System. This will initiate processing of test(s).

Results

Available results may be viewed or printed from the **Results** tab in the Results window on the NeuMoDx System touchscreen.

Test results are automatically generated by the NeuMoDx System software.

For quantitative assays, target concentration (log_{10} IU/ml) will be reported once a valid calibration has been implemented and a dynamic range has been established on the NeuMoDx System by the laboratory for the LDT.

For qualitative assays, a test result may be reported as Negative, Positive, Indeterminate, or Unresolved, based on the amplification status of the target and sample process control. The amplification status is determined based on cutoff parameters for real-time PCR curve analysis defined in the LDT ADF. Results are reported based on the decision algorithm in Table 1.

Table 1. NeuMoDx LDT DNA MM Test Strip Test Decision Algorithm

Result	Target	Sample Process Control (SPC1)	System Events
Positive	Amplified	N/A	No relevant errors
Negative	Not Amplified	Amplified	No relevant errors
Indeterminate	Not Amplified	Not Amplified	Relevant errors
Unresolved	Not Amplified	Not Amplified	No relevant errors

Quality Control

Clinical Laboratory Improvement Amendments (CLIA) regulations specify that the laboratory is responsible for implementing control procedures that monitor accuracy and precision of the complete analytical process, and must establish the number, type, and frequency of testing control materials using verified performance specifications for an unmodified, FDA-cleared or approved test system (42 CFR Part 493.1256).

1. External control materials must be validated by the lab for each assay performed. This includes the composition of controls, timing/frequency of running, and decision criteria around whether to invalidate a set of results due to (in)validity of controls. External controls are not provided by NeuMoDx Molecular, Inc.
2. The primers and probe for the detection of Sample Process Control 1 (SPC1) are included in the NeuMoDx LDT Master Mix, DNA. Monitoring detection of SPC1 allows the NeuMoDx System to monitor the efficacy of the DNA extraction and PCR amplification processes and appropriately qualify the results.

Invalid Results

If a test performed on the NeuMoDx System does not successfully process, it will be reported as either Indeterminate (IND) or Unresolved (UNR) based on the type of error that occurred.

An IND result will be reported if an instrument/system error is detected during sample processing. In the event an Indeterminate (IND) result is reported, a retest is recommended to obtain a valid result.

A UNR result will be reported if no target is detected and there is no amplification of the sample processing control, which indicates possible reagent failure or the presence of inhibitors. In the event a UNR result is reported, a retest is recommended to obtain a valid result.

Limitations

1. NeuMoDx LDT Master Mix, DNA can only be used on the NeuMoDx System and is not compatible with any other automated molecular diagnostic system. However, these test strips may be used in a manual process on any real-time PCR platform.
2. The performance of the NeuMoDx LDT Master Mix, DNA has ***only*** been validated using NeuMoDx model assays for bacterial DNA detection in urine and viral DNA detection in plasma. The performance characteristics of LDTs using this reagent are unknown and must be validated by the user's laboratory before diagnostic claims can be made.
3. Because detection of most pathogens is dependent on the number of organisms present in the sample, reliable results are dependent on proper specimen collection, handling, and storage.
4. Erroneous test results could occur from improper specimen collection, handling, storage, technical error, or sample mix-up. In addition, false negative results could occur because the number of organisms in the specimen is below the analytical sensitivity of the test.
5. The sample process control (SPC1) can be used as an indicator for system failure and inhibition and should be monitored for each test. Failure to do so may result in erroneous results.
6. The ability to use SPC1 as a monitor for inhibition needs be validated for each LDT by the lab prior to being used as a control or monitoring tool.
7. If the SPC1 does not amplify and the target result is Negative, a result of Indeterminate or Unresolved will be reported and the test should be repeated.
8. The end user must define and validate proper cut off criteria for each assay that is developed in order to have valid results.
9. Use is limited to personnel trained on the use of the NeuMoDx System.
10. Good laboratory practices, including changing gloves between handling patient specimens, are recommended to avoid contamination of specimens.

Quality Control

Clinical Laboratory Improvement Amendments (CLIA) regulations specify that the laboratory is responsible for implementing control procedures that monitor accuracy and precision of the complete analytical process, and must establish the number, type, and frequency of testing control materials using verified performance specifications for an unmodified, FDA-cleared or approved test system (42 CFR Part 493.1256).

1. External control materials must be validated by the lab for each assay performed. This includes the composition of controls, timing/frequency of running, and decision criteria around whether to invalidate a set of results due to (in)validity of controls. External controls are not provided by NeuMoDx Molecular, Inc.
2. The primers and probe for the detection of Sample Process Control 1 (SPC1) are included in the NeuMoDx LDT Master Mix, DNA. Monitoring detection of SPC1 allows the NeuMoDx System to monitor the efficacy of the DNA extraction and PCR amplification processes and appropriately qualify the results.

Performance Characteristics

Method

Performance characteristics of the NeuMoDx LDT Master Mix, DNA were determined by NeuMoDx Molecular, Inc. using a model DNA assay to demonstrate the NeuMoDx LDT DNA isolation and detection chemistry from plasma and urine specimens. In-house studies were performed on the NeuMoDx 288 Molecular System to determine both the analytical sensitivity of the assay when used in conjunction with NeuMoDx LDT Master Mix, DNA and the efficacy of the extraction process by extracting serial dilutions of the viral target in both matrices to characterize the linearity. Additional testing was then performed to demonstrate equivalent performance using the same model DNA assay to evaluate the NeuMoDx LDT DNA isolation and detection chemistry from plasma and urine specimens on the NeuMoDx 96 Molecular System.

The configurable portion of the Assay Definition File (ADF) determines all of the assay specific functions for an assay, including sample volume, real-time PCR profile, cut off criteria, result processing algorithms, and other function described in Table 2, below.

Table 2. NeuMoDx LDT Configurable Assay Definition File Parameters

LDT Configurable ADF Parameters			
Sample Volume	Ending Fluorescence Start Cycle	Peak Maximum Cycle	
Lysis Duration	Ending Fluorescence End Cycle	Minimum EP	
Ct Calling Algorithm	Fill Check Reporter	Real-time PCR	Activation
Result Processing Algorithm	Fill Check Threshold		Cool Down
Starting Fluorescence Start Cycle	Target Reporter		Cycling (X45)
Starting Fluorescence End Cycle	Peak Minimum Cycle		

References

1. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th edition. HHS Publication No. (CDC) 21-1112, Revised December 2009
2. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Fourth Edition. CLSI document M29-A4; May 2014

Symbols

The following symbols may appear in the instructions for use or on the packaging and labeling:

Symbol	Symbol definition
	Contains reagents sufficient for <N> reactions
	Use by
	In vitro diagnostic medical device
	Catalog number
	Batch code
	Manufacturer
	Temperature limit
	For prescription use only
	Authorized representative in the European Community

Symbol	Symbol definition
	Do not reuse
	CE Mark
	Consult instructions for use
	Warning
	Health Hazard
	Contains
	Contains biological material of animal origin
	Contains biological material of human origin
	Boric acid

Contact Information

For technical assistance and more information, please see our Technical Support Center at **support@qiagen.com**.

Technical support/Vigilance reporting: **support@qiagen.com**

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Ordering Information

Product	Cat. no.
NeuMoDx LDT Master Mix, DNA	100200
Related Products	
NeuMoDx Lysis Buffer 1	400400
NeuMoDx Lysis Buffer 2	400500
NeuMoDx Lysis Buffer3	400600
NeuMoDx Lysis Buffer 4	400700
NeuMoDx Lysis Buffer 5	400900
NeuMoDx Lysis Buffer 6	401700
NeuMoDx Cartridge	100100
NeuMoDx Extraction Plate	100200
NeuMoDx Wash Reagent	400100
NeuMoDx Release Reagent	400200
NeuMoDx LDT Primer/Probe Strip	100400
Hamilton CO-RE / CO-RE II Tips (300 µl) with Filters	235903
Hamilton CO-RE / CO-RE II Tips (1000 µl) with Filters	235905

For up-to-date licensing information and product-specific disclaimers, see the respective NeuMoDx kit handbook or operator manual. NeuMoDx kit handbooks are available at **www.neumodx.com** or can be requested from **support@qiagen.com** or your local distributor.

Document Revision History

Revision	Summary of Changes
A, 05/2022	Initial Release New Product Number (P/N 40600593) created for IVDR submission of General Reagents
B, 07/2023	Updated Emergo Address to Westervoortsedijk 60; 6827 AT Arnhem The Netherlands. Changed www.neumodx.com/client-resources to www.qiagen.com/neumodx-ifu.

Limited License Agreement for NeuMoDx LDT Master Mix, DNA

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the panel only. NeuMoDx grants no license under any of its intellectual property to use or incorporate the enclosed components of this panel with any components not included within this panel except as described in the protocols provided with the product, this handbook, and additional protocols available at **www.neumodx.com**. Some of these additional protocols have been provided by NeuMoDx users for NeuMoDx users. These protocols have not been thoroughly tested or optimized by NeuMoDx. NeuMoDx neither guarantees them nor warrants that they do not infringe the rights of third-parties.

2. Other than expressly stated licenses, NeuMoDx makes no warranty that this panel and/or its use(s) do not infringe the rights of third-parties.

3. This panel and its components are licensed for one-time use and may not be reused, refurbished, or resold.

4. NeuMoDx specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the panel agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. NeuMoDx may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the panel and/or its components.

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