

NxTAG[®] Gastrointestinal Pathogen Panel Package Insert

RUO For Research Use Only. Not for use in diagnostic procedures.

For use with the MAGPIX[®] System.

MLD-053-FKT-002 Rev A

03/2021

REF X053C0461

NxTAG Gastrointestinal Pathogen Panel (RUO)  96 TESTS

Technical Support

Telephone: 512-381-4397

North America Toll Free: 1-877-785-2323

International Toll Free: + 800-2939-4959

Email: support@luminexcorp.com












www.luminexcorp.com



Luminex Molecular Diagnostics, Inc.
439 University Ave.
Toronto, ON, Canada
M5G 1Y8

Symbols Glossary

You will encounter these symbols throughout this manual. They represent warnings, conditions, identifications, instructions, and regulatory agencies.

Symbol	Meaning	Symbol	Meaning
	Caution.		Use-by date.
	Batch Code.		Manufacturer.
	Contains Sufficient for <n> Tests.		Temperature Limit.
	Consult instructions for use.		Catalog(ue) Number.
	For Research Use Only. Not for use in diagnostic procedures.		Do not use if package is damaged.
	Keep Dry.		

Luminex Technical Support

Contact Luminex Technical Support by telephone in the U.S. and Canada by calling: 1-877-785-2323

Contact outside the U.S. and Canada by calling: +1 512-381-4397

International: + 800-2939-4959

Fax: 512-219-5114

Email: support@luminexcorp.com

Additional information is available on the website. Search on the desired topic, navigate through menus. Also, review the website's FAQ section. Enter <http://www.luminexcorp.com> in your browser's address field.

This manual can be updated periodically. To ensure that you have a current version, contact Technical Support.

Description

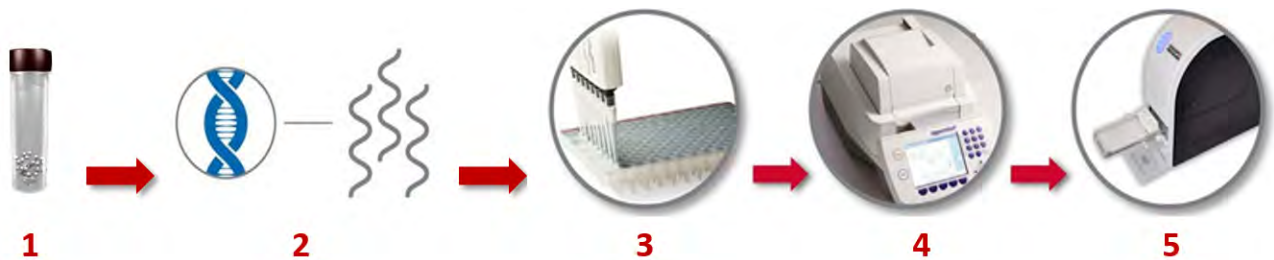
The NxTAG® Gastrointestinal Pathogen Panel (NxTAG® GPP) is a multiplexed nucleic acid test for the simultaneous qualitative detection and identification of multiple viral, bacterial, and parasitic nucleic acids in human stool samples or human stool in transport media. The following pathogen types, subtypes, and toxin genes are probed by the NxTAG GPP assay.

Bacteria
<i>Campylobacter</i> Group
<i>C. difficile</i> (Toxin A/B)
ETEC (LT/ST)
STEC (<i>stx1/stx2</i>)
<i>Shigella</i> spp. / EIEC
<i>Salmonella</i> spp.
<i>Vibrio cholerae</i>
<i>Yersinia enterocolitica</i>
Viruses
Adenovirus F40/41
Astrovirus
Norovirus GI/GII
Rotavirus A
Sapovirus GI, GII, GIV, GV
Parasites
<i>Cryptosporidium</i> Group
<i>Entamoeba histolytica</i>
<i>Giardia lamblia</i>

Principles of the Procedure

The NxTAG® Gastrointestinal Pathogen Panel (NxTAG GPP) incorporates multiplex Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) with the Luminex® proprietary universal tag sorting system on the Luminex platform to easily detect gastrointestinal pathogen targets. Extracted total nucleic acid is added to pre-plated, Lyophilized Bead Reagents (LBRs) and mixed to resuspend the reaction reagents. The reaction is amplified via RT-PCR and the resulting product undergoes near simultaneous bead hybridization within the sealed reaction well. The hybridized, tagged beads are then sorted and read on the MAGPIX® instrument. The generated signals are analyzed using the NxTAG GPP Assay File for SYNCT™ Software, providing a reliable, qualitative call for each of the 16 targets and internal control within each reaction well.

Figure 1: NxTAG® GPP Assay Workflow



Step 1	Pre-process by bead beating
Step 2	Nucleic acid extraction
Step 3	Load extracted nucleic acid to pre-plated test wells
Step 4	Multiplex RT-PCR and hybridization
Step 5	Data acquisition on MAGPIX® instrument

Assay Controls

- **Internal Control** - Bacteriophage MS2 is the internal control for the assay. This internal positive control is added to each sample prior to extraction. This internal control allows the user to ascertain whether the assay is functioning properly. Failure to detect the MS2 control indicates a failure at either the extraction step, the reverse-transcription step, or the PCR step. Low or no MS2 signal may be indicative of the presence of amplification inhibitors, which can lead to false negative results.
- **External Controls** - Good laboratory practice recommends running external positive and negative controls regularly. External controls should be used in accordance with local, state, federal accrediting organizations, as applicable.
- **Negative Amplification Control (No Template Control (NTC))** - The negative amplification control is DNase/RNase-free water.
- **Negative Extraction Control** - The negative extraction control is the lysis buffer added to the pretreatment tube (for raw stool samples) or the transport media (for stool in transport media) that has undergone the entire assay procedure, starting from pretreatment.

Materials Provided

The following table outlines reagents supplied in the kit and their storage conditions. Ensure the kit you are using is for NxTAG® Gastrointestinal Pathogen Panel.

Table 1. Reagents Supplied with the NxTAG® Gastrointestinal Pathogen Panel Kit

Reagents	Volume for 96 Tests	Storage Conditions
NxTAG® Gastrointestinal Pathogen Panel Plate	1 - 96-well plate containing 2 Lyophilized Bead Reagents per well	Store at 2°C to 8°C in the re-sealable pouch provided; avoid exposure to light and moisture.
MS2	1.5 mL x 2 vials	Store at -25°C to 8°C.
Foil Seals	8 pieces x 1 case	Store at 2°C to 30°C. Store at 15°C to 30°C after first use.

NOTE: Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not interchange kit components from different kit lots. Kit lots are identified on the kit carton label.

NOTE: The kit is shipped at 2°C to 30°C. Upon receipt, store the kit at 2°C to 8°C.

NOTE: To avoid exposing the NxTAG GPP plate to moisture, do not discard the desiccants included in the resealable pouch. Ensure the pouch is completely sealed after each use.

Software Accessory Package

- xPONENT® Data Acquisition Protocol for MAGPIX® instrument
- NxTAG® GPP Assay File for SYNCT™ Software

A Software Accessory Package (SAP) for use with this assay, containing the xPONENT data acquisition protocol and NxTAG GPP assay file, is available from Luminex Technical Support.

Once you have obtained the SAP from Luminex Technical Support, extract the files from the ZIP file. Ensure that the xPONENT data acquisition protocol is saved to a location that is accessible by the xPONENT Software, and that the NxTAG GPP assay file is saved to a location that is accessible by the SYNCT Software.

Materials Required but not Provided

Recommended Extraction Agents

Choose an extraction system from the list below. The associated reagents and consumables are also required.

bioMérieux® NucliSENS® easyMAG® System with Specific A protocol (bioMérieux Product No. 280140), and associated reagents and consumables

OR

bioMérieux EMAG System with Specific A protocol (bioMérieux Product No. 418591), and associated reagents and consumables

OR

Equivalent nucleic acid extraction method

bioMérieux NucliSENS easyMAG lysis buffer (Cat. # 280134 (4 x 1 L) or Cat # 200292 (48 x 2 mL))

OR

Appropriate lysis buffer for extraction system

Bertin® SK38 Soil Kit Bead Tubes (Bertin Cat. No. P000915-LYSK0-A or Luminex PN: GR032C0442)

Equipment and Consumables

- Computer with:
 - Operating System Microsoft® Windows® 7, 64-bit or Windows 10
 - PC Specifications as stated in the SYNCT™ Release Notes
 - SYNCT Software
- Luminex® instrument (NxTAG-enabled MAGPIX® instrument)
 - xPONENT® Software, calibrators, verifiers, controls, and Drive Fluid/Drive Fluid PLUS
 - NxTAG® Probe Adjustment Strip (Cat # C000Z0452)
 - Isopropanol (70%), 0.1N NaOH
 - Sonicator bath (Ultrasonic Cleaner, Cole-Parmer®, A-08849-00) or equivalent
- Optional for pretreatment step: Vortex 24-tube adapter (QIAGEN®, Cat# 13000-V1-24) or equivalent
- 20,800 RCF capable centrifuge with rotor capable of accommodating 1.5 to 2 mL microcentrifuge tube
- 96-well Non-Skirted Plate in a clear frame (Cat # C000Z0453) for thermal cyclers that are not compatible with fully-skirted plate
- Skirted Plate (Cat # C000Z0455) (96-well in white frame)
- Multichannel pipette or single channel pipette (20 µL to 1000 µL)
- PCR cooler rack (Eppendorf® 022510509) or equivalent
- DNase/RNase-Free Water
- Micronic Pierceable TPE Capmat Black (Cat. No. MP53087) or equivalent for thermal cyclers without adjustable lids
- Thermal Cycler

Replacement Materials (if needed)

NOTE: Full foil sheets can be purchased from 4titude, Catalog #: 4ti-0531.

- Foil Seals (Cat # C000Z0454) (8 pieces per case, each piece reseals 3 strips of 8-vessel/strip)

Warnings and Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. Do not eat, drink, smoke, or apply cosmetic products in the work areas.
3. Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free of DNases and RNases. Use only supplied or specified required consumables to ensure optimal test performance.
4. Train personnel who use, maintain, or clean the instrument in standard laboratory safety practices and follow those practices when handling the instrument.
5. Take care when handling, storing, and disposing of potentially infectious materials (i.e. samples, specimens, waste fluid, etc). Suitable barrier protection against potential pathogens is recommended during all stages of use. Adherence to appropriate local biosafety and biohazard guidelines or regulations is recommended when working with potentially infectious materials that may be unknown.
6. Handle waste disposal in accordance with accepted medical practice and applicable regulations. If spillage occurs immediately disinfect following appropriate laboratory procedures.
7. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Fresh clean gloves must be worn in each area and must be changed before leaving that area. Wash your hands thoroughly after performing the test.
8. Do not pipette by mouth.
9. For pre-analytical (sample extraction) steps, use the procedure that is provided with the sample extraction system.
10. Perform the procedure given in this package insert as described. Any deviation from the outlined protocols may result in assay failure or cause erroneous results.
11. Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not interchange kit components from different kit lots. Lot numbers are identified on the kit label.
12. Follow your institution's safety procedures for working with chemicals and handling biological samples.
13. In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.
14. Safety Data Sheets (SDS) are available by contacting Luminex Corporation or visiting our website at www.luminexcorp.com.

Assay Procedure

Collect, Transport, and Store Stool Samples

NOTE: Standard precautions should be taken with regard to sample collection, handling, and storage prior to extraction (refer to the latest edition of the CLSI MM13-A Guideline¹; and Farkas et al. (1996)²).

Place fresh stool samples in a sterile, leak-proof, wide-mouthed, preservative-free container or in a container with Cary-Blair or equivalent transport media. If collecting stool in a transport media ensure that a stool:medium ratio of 1:3 (1 part stool:3 parts medium) is obtained to dilute the stool 4x in the medium. NxTAG® GPP is not compatible with stool samples that have been stored in fixatives.

Transport and Store Raw Stool

Transport unpreserved raw stool samples in a sterile container to the laboratory on wet ice (2°C to 8°C) or dry ice (≤ -70°C).

Use NxTAG® GPP to test raw stool samples as soon as they are received in the laboratory. If not processed immediately, the stool samples can be stored refrigerated (2°C to 8°C) for up to two days or frozen ($\leq -70^{\circ}\text{C}$) until testing.

Luminex does not recommend repeat freezing and thawing of samples.

If desired, raw stool samples can be placed in Cary-Blair or equivalent holding media once received in the laboratory. To convert raw stool to stool in holding media, dilute the stool 4-fold with the media by adding 1 part raw stool to 3 parts media.

Transport and Store Stool in Transport Media

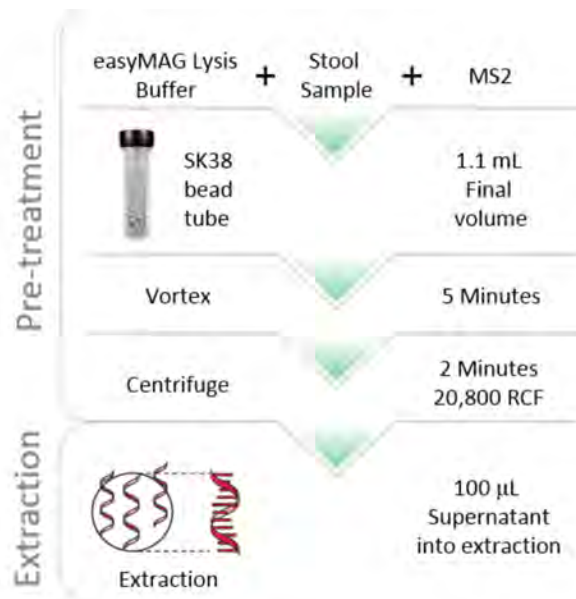
Transport stool samples in a Cary-Blair or equivalent transport media to the laboratory at room temperature (18°C to 25°C) or on wet ice (2°C to 8°C).

Use NxTAG® GPP to test stool samples in a Cary-Blair media as soon as they are received in the laboratory. If not processed immediately, the stools can be stored refrigerated (2°C to 8°C) for up to two days or frozen ($\leq -70^{\circ}\text{C}$) until testing.

Luminex does not recommend repeat freezing and thawing of samples.

Prepare Sample

Luminex recommends that nucleic acid be extracted from fresh or frozen stool samples. A pretreatment step consisting of bead beating in lysis buffer is required prior to nucleic acid extraction to ensure maximum extraction efficiency. Luminex recommends running at least one negative extraction control per batch of samples for pretreatment and extraction.

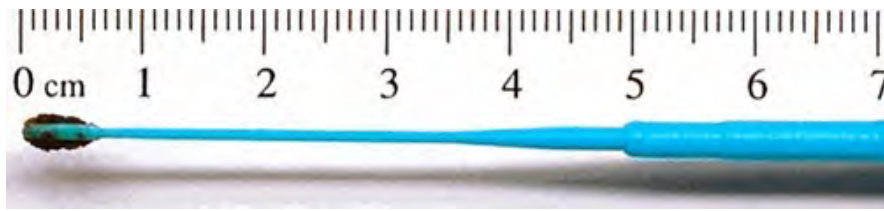


Pre-Treat Raw Stool

1. Add 1 mL of bioMérieux® NucliSENS® easyMAG® lysis buffer or appropriate lysis buffer for the extraction system to a Bertin® SK38 bead tube.
2. Add the raw stool sample to the bead tube containing the lysis buffer.

- a. For solid stool samples (typically Bristol types 1-5), add approximately 100 mg to 150 mg of stool to the bead tube. Luminex recommends that a 10 µL disposable inoculating loop be used to add the stool, filling the inside of the loop with stool.

Figure 2: Representative Amount of Solid Stool Input for Sample Treatment



NOTE: Adding too much stool may result in an increased PCR inhibition, while adding too little may result in low sensitivity.

- b. For raw liquid stool samples (typically Bristol types 6-7), add 100 µL of stool to the bead tube.
3. For the negative extraction control, add 100 µL of lysis buffer.
4. Add 20 µL of MS2 (provided) into all sample pretreatment tubes as an internal control. Luminex recommends not adding MS2 (internal control) to the negative extraction control.
5. Vortex the pretreatment tube for 5 minutes. If processing multiple samples, Luminex recommends using a vortex adapter.
6. Centrifuge the pretreatment bead tube at 20,800 RCF for 2 minutes to pellet any insoluble material.

Pre-Treat Stool in Transport Media

1. Add 700 µL of bioMérieux® NucliSENS® easyMAG® lysis buffer or appropriate lysis buffer for the extraction system to a Bertin® SK38 bead tube.
2. Mix the stool container to ensure a uniform stool mixture.
3. Add 400 µL of the stool/transport medium mixture to the bead tube containing the lysis buffer. For the negative extraction control add 400 µL of transport medium.
4. Add 20 µL of MS2 (provided) into all sample pretreatment tubes as an internal control. Luminex recommends not adding MS2 (internal control) to the negative extraction control.
5. Vortex the pretreatment tube for 5 minutes. If processing multiple samples, Luminex recommends using a vortex adapter.
6. Centrifuge the pretreatment bead tube at 20,800 RCF for 2 minutes to pellet any insoluble material.

Extract Nucleic Acid

1. Collect 100 µL of pretreatment supernatant from the middle of the tube; avoid aspirating the beads at the bottom of the tube or lipids from the top.
2. Load the 100 µL of pretreatment supernatant on to the bioMérieux® easyMAG® or EMAG® extraction system or equivalent nucleic acid extraction method, according to the manufacturer's instructions. If using easyMAG or EMAG, the pretreatment should be extracted using the protocol Specific A with a 110 µL elution volume.

NOTE: Pretreated supernatant (in the SK38 bead tube) can be stored at ≤ -70°C for up to 1 month.

Extract Nucleic Acid using the bioMérieux® easyMAG® and EMAG® Systems

The pretreatment should be extracted using the protocol Specific A with a 110 µL elution volume. NxTAG® GPP specific extraction parameters can be found in *Table 2* and *Table 4*.

NOTE: Refer to the manufacturer’s instructions for use of bioMérieux easyMAG and EMAG.

To configure the easyMAG, use these parameters:

Table 2. Parameter for the bioMérieux®_easyMAG®_System with Specific A Protocol

Page Name	Parameters	Settings
Define Extraction Request	Sample ID	Enter Sample ID
	Protocol	Specific A
	Matrix	Feces
	Volume	0.100 mL
	Eluate	110 µL
	Type	Primary
	Priority	Normal or High
Create Run (New Run window)	Run	Enter run name
	Workflow	Select: On-board Lysis Incubation, On-board silica incubation

Table 3. bioMérieux®_easyMAG®_System Silica Preparation and Addition

Extraction Step	Instructions
Silica Preparation	Dilute easyMAG® silica 1:1 in DNase/RNase free water
Silica Addition	Add 100 µL of diluted silica after on-board lysis incubation is complete, pipette mix five times at 1000 µL

To configure the EMAG for use with NxTAG® GPP, create an NxTAG GPP extraction protocol:

Table 4. Parameter for the bioMérieux® EMAG® System with Specific A Protocol

Tab Name	Parameters	Settings	
General	Extraction Method Name	Name the protocol. Example: (NxTAG GPP)	
	Description	Write a description of the protocol. Example: "Luminex protocol for NxTAG GPP sample extraction"	
Input	Off-board Lysis	Off (Do not select)	
	Matrices	Stool, others	
	Valid input volumes	List volume: 100 µL Default volume: 100 µL	
Preparation	Add these items to the Preparation protocol steps table in the indicated order	# Preparation protocol steps Details of selected step	
		1 Samples already prepared:	Do nothing
		2 Distribute reagent bottle to well:	Reagent Bottle: LB (lysis buffer) Volume: 2000 µL
		3 Incubate at room temperature:	Duration: 600 seconds
		4 Transfer silica to well:	Silica Name: Silica Volume: 50 µL
		5 Incubate at room temperature:	Duration 600 seconds
Extraction	Extraction Protocol	Specific A	
	Valid Elution Volume	List volume: 110 µL, Default volume: 110 µL	
Eluate Transfer	-	Select: Keep eluates in vessel	
Status	-	Activated	

NOTE: The extracted nucleic acid can be stored at ≤ -70°C for up to one month, if not tested immediately.

Program and Preheat the Thermal Cycler

NOTE: Perform PCR setup in the pre-PCR area.

Program the following PCR protocol into the thermal cycler with a heated lid (105°C), and pre-heat the thermal cycler to 42°C prior to plate setup:

Figure 3: PCR and Hybridization Conditions



Thermal Cycler	Rate Settings
Eppendorf® Pro S or EP gradient S	75% (~4.5°C/s)
Bio-Rad® C1000 series (Deep well module)	2.5°C/s
ABI® Veriti	Max (~3.5°C/s with normal block)
ABI® 9700	9600 emulation mode (~1.6°C/s)

The total thermal cycling run time for NxTAG® Gastrointestinal Pathogen Panel should range between approximately 2 hours 15 minutes and 2 hours 45 minutes.

Setup the NxTAG® GPP Reaction Plate

NOTE: Pre-heat the thermal cycler to 42°C prior to plate setup.

NOTE: Perform PCR setup in the pre-PCR area.

1. If frozen, thaw the extracted nucleic acid samples on ice. Briefly vortex the samples followed by a quick spin to collect the samples to the bottom.
2. Place samples on a chilled PCR cooler block or equivalent.
3. Remove the assay plate from its storage pouch. Place the required number of vessels into the appropriate PCR setup plate (e.g., skirted plate for thermal cyclers with adjustable lids and non-skirted plate for thermal cyclers without adjustable lids).

NOTE: Luminex recommends the first sample be placed in location A1.

- a. Firmly press down on the strips to snap into place, ensuring they are flush with the plate surface.
- b. Return unused vessels to the pouch, seal, and store at recommended storage conditions.

NOTE: Protect the assay plate from prolonged light exposure.

4. Tap the plate on the benchtop to ensure the Lyophilized Bead Reagents (LBRs) are at the bottom of the vessel.
5. Place the plate on a chilled PCR cooler block or equivalent.
6. Use the end-tabs to peel the clear release liner.

NOTE: Do not touch the black adhesive.

7. Dispense 35 µL of sample or control to each PCR vessel, by using the pipette tip to pierce the foil at an angle.

- a. Insert the tip a third to halfway down into the vessel.
 - b. Dispense the sample into the vessel and wait 1 to 2 seconds while maintaining the pipette tip inside the vessel.
 - c. Push the tip all the way to the bottom of the vessel and pipette up and down at least three times to reconstitute the LBRs.
8. Reseal the plate after the sample addition using the pre-cut strips of foil provided. Apply the foil(s) directly on top of the plate and press firmly on and around the wells to ensure a tight seal.

NOTE: Ensure the foil covers the wells and surrounding black adhesive.

NOTE: Do not vortex and spin down the plate.

Run Thermal Protocol

1. Place the foil-sealed plate in the pre-heated thermal cycler and run the protocol.
2. If using a thermal cycler without an adjustable lid, place a micronic pierceable TPE Capmat black or equivalent on top of the sealed plate.

Setup System Software

Import the Data Acquisition Protocol into xPONENT® Software

NOTE: Please refer to the applicable user manual. Ensure the NxTAG® Gastrointestinal Pathogen Panel data acquisition protocol is saved to a location that is accessible by the xPONENT® Software on the MAGPIX® computer.

If the appropriate protocol is already installed on the computer that controls the Luminex® instrument where the assay is being run, skip the following steps:

1. Log into xPONENT Software.
2. Navigate to the **Protocols** page > **Protocols** tab.
3. Click **Import**.
4. In the **Open** dialog box, browse to the folder where the NxTAG Gastrointestinal Pathogen Panel data acquisition protocol is located, and choose the **NxTAG GPP 24-plex[1].lxt2** protocol file. Click **Open**.
5. In the **Imported Protocol File** dialog box, click **OK**. The imported protocol is displayed in the **Installed Protocols** section.

Configure MAGPIX® for Data Acquisition

Prepare the System

NOTE: Please refer to the applicable user manual for software requirements, setup, calibration and verification, and troubleshooting.

NOTE: When setting up xPONENT®, ensure that the Use US regionalization format only option is selected in Admin > CSV Options.

NOTE: Make sure you are using a NxTAG®-enabled MAGPIX® instrument.

1. Log into the xPONENT Software.
2. Perform the **Enhanced Startup Routine** at least once a week along with the required probe sonication.
3. Adjust the sample probe height at least once a week, or as needed.

- a. When adjusting the sample probe height, use the same plate type that will be used when running the NxTAG GPP assay plate. Use either the skirted plate or the non-skirted plate (if using a thermal cycler without an adjustable lid) with the NxTAG Probe Adjustment Strip and one alignment sphere.

NOTE: Probe height must be re-adjusted if changing between skirted and non-skirted plates.

- b. Save probe height adjustments as **NxTAG Assay Plate**. If prompted to over-write the existing results, click **Yes**.

NOTE: For more information on adjusting the sample probe height, refer to the applicable user manual.

4. Navigate to the **Maintenance** page > **Probe & Heater** tab.
5. Select **ON** under **Plate Heater** and enter **37** in the **Set Temperature** field to heat the MAGPIX® heater plate to 37°C. Click **Apply**.
6. Navigate to the **Maintenance** page > **Cmnds & Routines** tab. Click **Eject**. Add the appropriate reagents to the off-plate reagent reservoirs, as specified by the **Post-Batch Routine** indicated in the software. Click **Retract**.

NOTE: The Post-Batch Routine is included in the assay protocol.

Create Batch in xPONENT® Software

1. Navigate to the **Batches** page > **Batches** tab > click **Create a New Batch from an Existing Protocol**.
2. Choose the **NxTAG GPP 24-plex** protocol in the **Select a Protocol** list.
3. Click **Next**. Select the appropriate wells where the samples will be analyzed and then click **Unknown**. The selected wells are highlighted.
4. Click **Import List** to import a sample list or enter the appropriate Sample ID for each well. Do not change the default **Dilution** settings.

NOTE: The Sample ID name cannot be duplicated within a Run. Each sample **MUST** have a unique ID. If you are running replicates or running the same control sample more than once, please make sure you assign a unique Sample ID, for example, by assigning "-1" or "-2" to the end of the proposed Sample ID.

5. Click **Save**. The batch is now saved as a pending batch and ready to run.
6. If running multiple batches on the same plate, create a Multi-Batch.

Create a Multi-Batch in xPONENT® Software

The Multi-batch feature automatically sets the batches side-by-side if space remains on the plate. Ensure that the batches fit on one plate. If space limitations create an overlap, an error message displays. Results for each batch are saved as individual batch files. Batches must be created first, before they can be combined on one plate to create a multi-batch.

NOTE: There is a limit of 96 batches in a multi-batch.

NOTE: You cannot add a batch that forces multiple plates to a multi-batch operation. All batches must use the same plate name.

1. Navigate to the **Batches** page > **Batches** tab > click **Create a New Multi-Batch**. The **New Multi-Batch** subtab displays.
 - a. If the **Select Pending Batch** dialog box displays, choose the batch you want to add to the new multi-batch list.
 - b. Click **OK**.
2. Click **Add** to add a batch. The **Select Pending Batch** dialog box displays.
3. Choose a batch from the available options, including batches newly created.
4. Click **OK**. The selected batch will then display on the plate layout.

NOTE: After you add each batch, the software automatically adds the next batch to the first well of the next column or row (depending on the plate direction). You can also select a well first, which places the next batch in your chosen location.

NOTE: If the batches chosen do not fit on the plate, a **Multi-Batch Error** dialog box opens, indicating you must edit one or more of the selected batches.

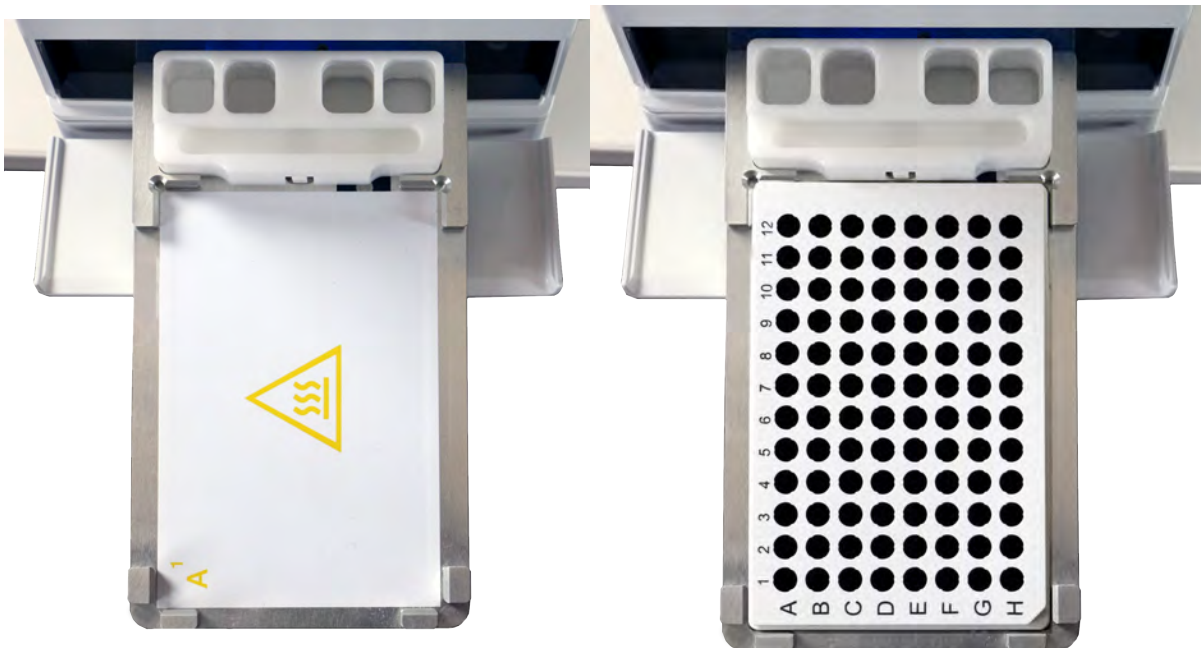
Acquire Data

Run Batch in xPONENT® Software

1. Navigate to the **Batches** page > **Batches** tab. Choose the pending batch that you want to run.
2. Upon completion of thermal cycling, click **Eject** to place the assay plate on the prepared MAGPIX heater block. Click **Retract** to retract the holder.

NOTE: Be sure to leave the seal in place.

NOTE: When placing the plate on the heater block, ensure that the numbers are on the left side and the letters are closest to you.



3. Click **Run** to start acquisition.
4. Verify the information in the warning dialog boxes and click **OK**.

Complete Run in xPONENT® Software

1. When the run is complete, navigate to the **Home** page > **Probe and Heater** tab.
2. Select **OFF** to turn off the heater and click **Eject** to remove the plate from the heater block. Then, click **Retract**.
3. Carefully discard the test strip(s) into a biohazard bag, sealing the bag to avoid aerosolization of the amplicons.
4. If re-using the plate, clean by soaking in a 10% household bleach solution for 15 minutes.
5. Rinse the plate under running tap water to remove the bleach, and air dry on paper towels or wipe with a cloth soaked in 70% alcohol for fast drying, if necessary.

Setup SYNCT™ Software


Install the NxTAG® Module in SYNCT™ Software for the First Time

Ensure that the SYNCT™ Software is on your computer with the NxTAG® module installed. If SYNCT Software is not installed, or the NxTAG module is not installed, then follow the procedures in the *SYNCT Installation Instructions*.

Import the Assay File into SYNCT™ Software

NOTE: Ensure the NxTAG® GPP assay file is saved to a location that is accessible by the SYNCT™ Software.

If you have already imported the correct version of the NxTAG® GPP assay file into SYNCT (Assay code: NGPA), skip the following steps:

1. Start the SYNCT Software and login with the appropriate SYNCT user.
2. Click  in the upper left-hand corner of the screen and navigate to **Assay Management > Assay Management** page.
3. Click **Import Assay** from the Page Action bar at bottom of the page. The **Import File** window displays.


NOTE: Do NOT double click. SYNCT™ Software requires one click when navigating to the correct file location.

- a. Choose the **Devices** and the **Files**.
- b. Choose the location under **Files** to locate **NxTAG GPP_RUO_NGPA_A.assay** to import, the file name will populate in the **File Name** field.
- c. Click **OK**.

Define Controls and Test Panels


Define a Negative Amplification Control (No Template Control) in SYNCT™ Software

To define a negative amplification control in SYNCT™ Software, complete the following:

1. Click  in the upper left-hand corner of the screen and navigate to **Assay Management > Controls** page.
2. Click **New Control** from the Page Action bar at bottom of the page.
3. In the window that displays, complete the following:
 - a. Enter the control **Name** (Required) and **Manufacturer** (Optional) information.
 - b. Choose the **NxTAG GPP RUO** assay in the **Assay** field with the corresponding assay code and version.
 - c. Click in the **Expected Results** (Required) field. The **Expected Results** window displays.
 - i. Set the expected result for all tests to **Negative** by selecting the **All Negative** check box.
 - ii. Click **Close**.
 - d. Click **Save**. The newly defined control displays in the **Controls** window.

Define a Negative Control in SYNCT™ Software

To define a negative control in SYNCT™ Software, complete the following:


1. Click  in the upper left-hand corner of the screen and navigate to **Assay Management > Controls** page.
2. Click **New Control** from the Page Action bar at bottom of the page.
3. In the window that displays, complete the following:
 - a. Enter the control **Name** (Required) and **Manufacturer** (Optional) information.
 - b. Choose the **NxTAG GPP RUO** assay in the **Assay** field with the corresponding assay code and version.
 - c. Click in the **Expected Results** (Required) field. The **Expected Results** window displays.
 - i. Set the expected result for all tests to **Negative** by selecting the **All Negative** check box.

NOTE: If the internal control was added to the negative, select Positive as the expected result for the internal control.
 - ii. Click **Close**.
 - d. Click **Save**. The newly defined control displays in the **Controls** window.

Define an External Positive Control in SYNCT™ Software

NOTE: Name the controls the same name as the controls in xPONENT®, so the control will be automatically defined in the SYNCT™ Software.

To define a external positive control in SYNCT Software, complete the following:


1. Click  in the upper left-hand corner of the screen and navigate to **Assay Management > Controls** page.
2. Click **New Control** from the Page Action bar at bottom of the page.
3. In the window that displays, complete the following:
 - a. Enter the control **Name** (Required) and **Manufacturer** (Optional) information.
 - b. Choose the **NxTAG GPP RUO** assay in the **Assay** field with the corresponding assay code and version.
 - c. Click in the **Expected Results** (Required) field. The **Expected Results** window displays.
 - i. For tests that are known to be positive in the sample, set the expected result to **Positive**.
 - ii. For tests that are known to be negative in the sample, set the expected result to **Negative**.
 - iii. If the expected result is unknown for a particular test, select **NA (No Analysis)**.
 - iv. Click **Close**.
 - d. Click **Save**. The newly defined control displays in the **Controls** window.

Define Test Panels in SYNCT™ Software

For each Order in SYNCT™ Software, you can choose whether a test result is Selected or Masked. Masked test results will not be reported for that sample. If certain subset of tests is ordered regularly, you can pre-define a Test Panel to make the ordering process easier. Then you can select the appropriate Test Panel when editing the Order instead of selecting or masking individual tests.

A default Test Panel that has all the tests selected is provided with the assay.

To define a Test Panel in SYNCT Software within the assay, complete the following:

1. Click  in the upper left-hand corner of the screen and navigate to **Assay Management > Assay Management** page.
2. Choose the **NxTAG GPP RUO** assay.
3. Click **Assay Options** from the Page Action bar at bottom of the page. The **Assay Options** window displays.

- a. Click the **Test Panels** tab at the top of the window.
- b. Click the **New Panel** button to create a new **Test Panel**. The new **Test Panel** displays within the **Test Panels** section.
- c. By default, all tests are **Selected** for the **Test Panel**. Create a custom **Test Panel** by clicking the **Masked** setting for the appropriate test(s).

NOTE: Tests with Masked settings chosen will not have test results reported.


- d. Click **Save Changes**.
- e. In the **Messages** dialog box that displays, click **OK**.

Analyze Results in SYNCT™ Software

Create Run from Imported Raw Data in SYNCT™ Software

The Import Raw Data function allows a raw data (CSV) file from xPONENT® Software to be imported.

To manually import the xPONENT raw data into the SYNCT Software, complete the following:

1. Click  in the upper left-hand corner of the screen and navigate to **NxTAG > Runs** page.
2. Click **Import Raw Data** from the Page Action bar at bottom of the page. The **Import xPONENT Data** window displays.

NOTE: Do NOT double click. SYNCT Software requires a single click when navigating to the correct file location.

- a. Choose the **Location** and the **Files**.
- b. Choose the batch file. The **Run Name** field is automatically populated with the Batch name from the xPONENT file.

NOTE: By default, the Run Name is the same as the batch name imported from the xPONENT file.


- c. Click **OK**. Orders are created for all samples within the imported batch file and can then be edited in SYNCT.

Edit and Review Orders in SYNCT™ Software

After the batch data is imported, an Order is created for each of the samples in the batch file. Review and edit the Orders prior to analyzing the Run.

NOTE: The Sample ID name cannot be duplicated within a Run. Each sample **MUST** have a unique ID. If you are running replicates or running the same control sample more than once, please make sure you assign a unique Sample ID, for example, by assigning “-1” or “-2” to the end of the proposed Sample ID.

Select multiple orders of the same sample type (sample or control) and edit them at the same time. This is useful when entering kit lot information for all sample orders at the same time, or for applying a Test Panel to multiple orders at the same time. Complete the following in SYNCT™ Software:

1. Click  in the upper left-hand corner of the screen and navigate to **NxTAG > Runs** page.
2. Click the “+” sign next to the Run that contains the samples to edit.
3. Select the sample(s) to edit.
4. Click **Edit Orders** from the Page Action bar at bottom of the page.
5. In the window that displays, edit the following information:

- For **Samples**:
 - i. From the **Sample Type** drop-down menu, choose **Sample**.
 - ii. If allowed, from the **Test Panels** drop-down menu, choose the appropriate Test Panel OR customize any of the tests listed by clicking **Selected** or **Masked**.
 - iii. Update the name of the sample in the **Sample ID** field (Available if a single Order is selected for editing).
 - iv. Optionally, include any necessary information in the **Accession ID** and **Requisition Number** fields.

NOTE: Depending on the SYNCT settings, the Accession ID and Requisition Number may not be visible or you may not have to enter any information within those fields.
 - v. Optionally, enter the kit lot number in the **Kit Lot Number** field.

NOTE: Kit lot numbers are 11 digits separated by a dash. Do not omit the dash when entering the number.

NOTE: If you enter a Kit Lot Number, you will be required to enter a Lot Expiration date.
 - vi. Optionally, click the calendar icon in the **Kit Lot Expiration** field to set the lot expiration date.


NOTE: Use information provided with your kit for the Kit Lot Number and Kit Lot Expiration.
 - vii. Click **OK**.
- For **Control**:
 - i. From the **Sample Type** drop-down menu, choose **Control**.
 - ii. Click to choose a pre-defined control to be applied.
 - iii. Enter the name of the control in the **Sample ID** field. (Available if a single Order is selected for editing.)
 - iv. Optionally, enter the kit lot information in the **Kit Lot Number** field.
 - v. Optionally, click the calendar icon in the **Kit Lot Expiration** field to set the lot expiration date.

NOTE: Use information provided with your kit for the Kit Lot Number and Kit Lot Expiration.
 - vi. Click **OK**.

Process Run in SYNCT™ Software







NOTE: Prior to proceeding, confirm that all Orders to be processed are correct. Once you proceed, you cannot change the sample type, or expected control results, after the test has been analyzed.

To process the Run in SYNCT™ Software, complete the following:

1. Click  in the upper left-hand corner of the screen and navigate to **NxTAG > Runs** page.
2. Select the Sample ID (Run) to process.
3. Click **Process Run** from the Page Action bar at bottom of the page. A dialog box displays, “**Confirm all orders are correct before proceeding. Do you want to continue?**”.
4. Click **Yes** to proceed with processing the Run.
5. Once the Run has completed processing, the Run is removed from the **NxTAG Run** view. The results of the Run can be found by clicking the **Results** icon from the **System Navigation Menu** and locating the processed Run from the list.

Result Call Definitions

For a general description of Results page functionality, please refer to the SYNCT™ *Software User Manual*.

1. Click  in the upper left-hand corner of the screen and navigate to **Results > Results** page.
2. Click the “+” sign next to the Run Results you want to see a Status for.
 - The **Status** column indicates whether there are Errors, Warnings, Info messages, or user comments for a sample. Click the  in the **Status** column to display the messages in the sample row. The **Status** column will display a  if a sample has an error. If there are no messages for the sample, the  will not appear.
 - The **Alert** column indicates if any test has a positive result. If the result is positive the **Alert** column will display a  for that sample.
 - The **Alert** column indicates if a control has failed. If the control failed, the **Alert** column will display a red exclamation mark for that control.
 - The **Result** column displays the summary result for the sample. To see individual results for each test, click  next to the summary results in the **Result** column. The results are shown grouped by result type in the sample row.

The following results can appear for samples:

Result Column	Meaning
Invalid	Any target that has an invalid result. Some targets may have valid positive or negative results. Expand the Result column to see the results for the individual targets.
Target1 Positive, Target2 Positive Target3 Positive	The specified target has a positive result. A maximum of three positive targets will be listed.
Positive Detected	More than three targets have a positive result.
Negative	All targets are negative.

The following results can appear for controls:


Result Column	Meaning
Pass	All target results match the expected results.
Fail	Any target result does not match the expected result or the target is set to Invalid. Expand the test column to see the results for the individual tests.
Invalid	If all negative controls failed due to instrument error or well not read, then the positive control will be Invalid.

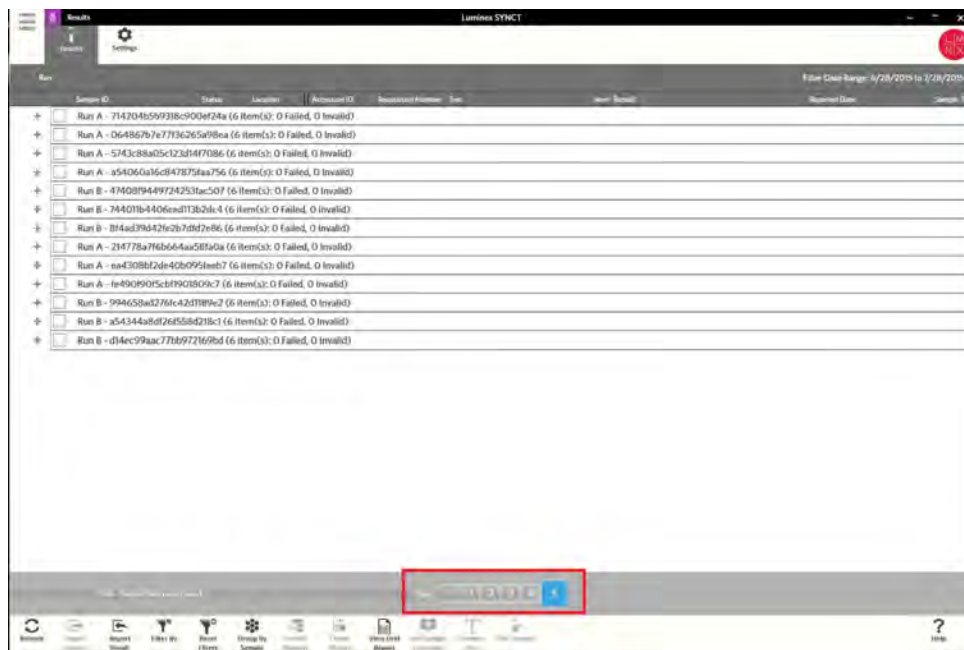
Report Type Definitions

The following reports are available for the NxTAG® Gastrointestinal Pathogen Panel assay:

Report Title	Summary of Contents
Sample Summary	Shows the result for each target for a sample.
Sample Details	Shows the result, calculated signal value, and threshold used to determine the result for each target for a sample.
Control Summary	Shows the expected result, and pass or fail result, for each target for a control.
Control Details	Shows the expected result, pass or fail result, and calculated signal for each target for a control.
Run Report	Shows a summary result for each sample that includes all positive tests.
Run Details	Contains a Run summary, Sample Details for each sample, and details for each selected target.


View Results in SYNCT[™] Software

1. Click  in the upper left-hand corner of the screen and navigate to **Results > Results** page.
2. When there are multiple pages of results in SYNCT[™] Software, page arrows and numbers will display at the bottom of the screen. Click the left and right arrows to scroll through the pages of results or, if you know what page the results are on, click the page number.



Create and Print a Report in SYNCT[™] Software

To create a report, complete the following:

1. Click  in the upper left-hand corner of the screen and navigate to **Results > Results** page.
2. Select the Run or samples that the report is to be generated for.

3. Click **Create Report** from the Page Action bar at bottom of the page. The **Generate Reports** window displays.
NOTE: You can select one sample in order to view the report, however the report may have results from other samples in it. You can also export to a chosen location and print the report.
4. Choose the type of report to be created from the options provided. The report displays in a separate window.
NOTE: Reports generated can have a customized header.
5. In the Report window, click **Print Report** to print the report. The **Print** dialog box will display.
 - a. Choose the printer and print settings, then click **Print**.

Troubleshoot

Re-Test Recommendations Prior to Data Acquisition

Thermal Cycler Error: If an error in the thermal cycler program is noticed after a particular step is initiated, re-test the samples.

Re-Test Recommendations After Data Acquisition

Under certain circumstances, data analysis software will generate a target call of "Invalid" with associated error message(s) for one or more samples in a plate. These scenarios are summarized (with re-test recommendations) below.

Re-Test Recommendations for Invalid Internal Control

If the SYNCT™ Software indicates a result of 'Invalid' with a message '*Internal Control failed*', the specific signal for the MS2 internal control is below the positive call threshold. When an *Invalid* internal control result is obtained, targets that are positive will be reported as *Positive*, targets that are negative will be reported as *Invalid*.

There are six probable causes of '*Invalid*' internal control results:

1. The stool sample contains traces of PCR inhibitors that were carried through the extraction process.
Recommended Action:
 - a. Prepare a 1/5 dilution of the extracted nucleic acid (in elution buffer) and re-test with NxTAG® GPP.
 - b. If the internal control is reported as a PASS in the sample re-run after 1/5 dilution of the nucleic acid, report assay results.
 - c. If the internal control is reported as a FAIL in the sample re-run after 1/5 dilution of the extract, re-extract the stool sample and re-run with NxTAG GPP.
 - d. If the internal control is still reported as a FAIL upon re-retraction and Re-run, report the results as INDETERMINATE.
2. No MS2 was added into the sample at the extraction step.
Recommended Action: Re-extract the sample. Prepare a new pretreatment ensuring MS2 has been added.
3. Failure to fully resuspend the Lyophilized Bead Reagents, or insufficient sample was added during the reaction setup.
Recommended Action: Retest starting from the extracted nucleic acid. Ensure that the Lyophilized Bead Reagents are at the bottom of the vessel by gently taping the assay plate on the benchtop and that the added sample volume is 35 µl.

4. Thermal cycler error.
Recommended Action: Retest starting from the extracted nucleic acid. Ensure the correct thermal cycling profile has been programmed.
5. Sample extraction failure, or insufficient sample was added during extraction.
Recommended Action: Re-extract the sample starting from the pretreatment. Ensure that the correct volumes are loaded onto the extraction system.
6. Low Bead Count: An insufficient number of beads were aspirated by the MAGPIX® instrument or the beads aggregated in the instrument, preventing an accurate count.
Recommended Action: Sonicate and clean the MAGPIX sample probe. Ensure the enhanced startup routine and post-batch cleaning routines are being performed.

Re-test Recommendations for Invalid Results

Scenarios are summarized (with re-test recommendations) in the table below.

Table 5. Invalid Results

Software Result and Messages	Problem	Possible Cause(s)	Recommendation(s)
<p><i>Result:</i> Invalid</p> <p><i>Message:</i> "Internal Control failed."</p>	Refer to Re-Test Recommendations for Invalid Internal Control.		
<p><i>Result:</i> Invalid</p> <p><i>Message:</i> "<Target Name>: non-specific signal detected in control sample"</p>	An unexpected target was detected in a control sample.	Contamination may have occurred during extraction, with extraction reagents, during sample addition, or the internal control was added to the negative extraction control.	<ol style="list-style-type: none"> 1. Clean all work surfaces and equipment with a 10% household bleach solution or other products (such as peroxide wipes) that are capable of degrading DNA amplicon contamination. Use fresh gloves and lab coats and follow a direction workflow to avoid introducing post-PCR amplicon contamination. 2. Re-extract the stool samples, starting with a new pretreatment, including the negative extraction control, with new (un-used) reagents.
<p><i>Result:</i> Invalid</p> <p><i>Message:</i> "Run failed. All negative control samples have failed"</p>	An instrument error occurred and all samples identified as negative controls are invalid.	Refer to the applicable user manual for possible causes.	Re-run the extracted nucleic acid samples.

Software Result and Messages	Problem	Possible Cause(s)	Recommendation(s)
<p><i>Result:</i> Invalid</p> <p><i>Message:</i> "<Target Name>: invalid value encountered" OR <Target Name>: low bead count"</p>	<p>The sample probe failed to acquire enough of the sample.</p>	<p>Sample probe height adjustment was not completed successfully.</p> <p>Failed to fully re-suspend Lyophilized Bead Reagents.</p> <p>An insufficient number of beads were aspirated by the MAGPIX® instrument or the beads aggregated in the instrument, preventing an accurate count.</p>	<p>1. Sonicate and clean the MAGPIX sample probe. Ensure the enhanced startup routine and post-batch cleaning routines are being performed.</p> <p>2. Re-run the extracted sample after repeating the sample probe height adjustment procedure. Ensure the Lyophilized Bead Reagents are at the bottom of the vessel by gently tapping the assay plate on the benchtop and that the added sample volume is 35 µL.</p>
<p><i>Result:</i> Invalid</p> <p><i>Message:</i> "<Target Name>: invalid negative control value"</p>	<p>Failed to acquire enough of target signal within all negative control samples.</p>	<p>Sample probe height was not completed successfully.</p> <p>Failed to fully re-suspend Lyophilized Bead Reagents.</p> <p>An insufficient number of beads were aspirated by the MAGPIX instrument or the beads aggregated in the instrument, preventing an accurate count.</p>	<p>1. Sonicate and clean the MAGPIX sample probe. Ensure the enhanced startup routine and post-batch cleaning routines are being performed.</p> <p>2. Re-run the extracted sample after repeating the sample probe height adjustment procedure. Ensure the Lyophilized Bead Reagents are at the bottom of the vessel by gently tapping the assay plate on the benchtop and that the added sample volume is 35 µL.</p>

Software Result and Messages	Problem	Possible Cause(s)	Recommendation(s)
<p><i>Result:</i> Invalid</p> <p><i>Message:</i> "Inconclusive results based on abnormal signals"</p>	Background cannot be calculated as multiple targets have abnormal signals.	Contamination may have occurred during extraction, during sample addition, or instrument failure.	<ol style="list-style-type: none"> 1. Clean all work surfaces and equipment with a 10% household bleach solution or other products (such as per-oxide wipes) that are capable of degrading DNA amplicon contamination. Use fresh gloves and lab coats and follow a direction workflow to avoid introducing post-PCR amplicon contamination. 2. Re-extract the stool samples, starting with a new pre-treatment, including the negative extraction control with new (un-used) reagents.
<p><i>Result:</i> Invalid</p> <p><i>Message:</i> "This well was not read by the Luminex instrument."</p>	No signal is detected.	Instrument failed or user terminated during data acquisition or extraction failure.	Re-run the extracted nucleic acid sample.
<p><i>Result:</i> Fail</p> <p><i>Message:</i> "Control failed: <Target name> result did not match expected result" OR "<Target Name>: non-specific signal detected"</p>	Unexpected target call in the control.	Wrong control samples were used or Extraction failure or error occurred during extraction or sample addition.	Re-extract and re-run the sample.
<p><i>Result:</i> Invalid</p> <p><i>Message:</i> "Internal Control failed."*</p>	Low Bead Count	An insufficient number of beads were aspirated by the MAGPIX® instrument or the beads aggregated in the instrument, preventing an accurate count.	<ol style="list-style-type: none"> 1. Sonicate and clean the MAGPIX sample probe. Ensure the enhanced startup routine and post-batch cleaning routines are being performed. 2. Re-run the extracted nucleic acid sample.

NOTE: * 'Invalid' internal control results with low bead count for Internal Control for ALL samples run on the same plate indicates that the wrong assay plate may have been run. NxTAG® RPP (+/- SARS-CoV2) and NxTAG® GPP have different bead regions for the internal control. If NxTAG® RPP (+/- SARS-CoV2) was inadvertently run in place of NxTAG® GPP, all samples would contain an invalid internal control call due to low bead count.

Recommended Action: Rerun the samples from the extracted nucleic acid using an NxTAG® GPP assay plate and appropriate acquisition file.

References

1. MM13-A Collection, Transport, Preparation and Storage of Specimens for Molecular Methods; Approved Guideline, 1st Edition.
2. Specimen collection and storage for diagnostic molecular pathology investigation. Farkas DH, Kaul KL, Wiedbrauk DL, Kiechle FL Arch Pathol Lab Med. 1996 Jun;120(6):591-6.

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www.rafer.es

Barcelona ☎ 93 645 50 28 ✉ barcelona@rafer.es	Bilbao ☎ 94 499 85 80 ✉ bilbao@rafer.es	La Coruña ☎ 981 93 89 26 ✉ galicia@rafer.es	Madrid ☎ 91 365 15 70 ✉ madrid@rafer.es	Málaga ☎ 639 359 792 ✉ malaga@rafer.es	Sevilla ☎ 954 369 334 ✉ sevilla@rafer.es	Valencia ☎ 96 340 48 00 ✉ levante@rafer.es	Zaragoza ☎ 976 23 74 00 ✉ rafer@rafer.es	Lisboa ☎ 21 154 19 98 ✉ lisboa@rafer.es
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