HANDBOOK

Nucleo-pore® Stool DNA Mini Kit

☐	**Nucleo-pore® Stool DNA Mini Kit**	NP-7011D	50 Preps
☐	**Nucleo-pore® Stool DNA Mini Kit**	NP-7012D	250 Preps

www.genetixbiotech.com

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COMPONENTS

Kit contents

Nucleo-pore® Stool DNA Mini Kit

Cat. No.	NP-7011D	NP-7012D
Number of Preps	**50 preps**	**250preps**
Lysis Buffer FL1	50 mL	2 × 125 mL
Lysis Buffer FL2	10 mL	50 mL
Binding Buffer FB	60 mL	2 × 125 mL
Wash Buffer FWB1	30 mL	2 × 75 mL
Wash Buffer FWB2 (Concentrate)*	25 mL	1 × 25 mL 1 × 50 mL
Elution Buffer FEB**	13 mL	30 mL
NP Bead Tubes Type A	50	250
Nucleopore® Inhibitor Removal Columns (red rings)	50	250
Nucleopore® DNA Stool Columns (green rings)	50	250
Collection Tubes (2 mL)	50	250
Collection Tubes (2 mL, lid)	50	250
User manual	1	1

* Please see “Preparation & storage of Reagents”

** Composition of Elution Buffer FEB: 5 mM Tris/HCl, pH 8.5

Reagents, consumables, and equipment not provided with the kit

- 96 – 100% ethanol
- Beta-mercaptoethanol to 0.5%(v/v)
- 1.5 ml microcentrifuge tubes
- Disposable pipette tips
- Manual pipetts
- Centrifuge
- Vortex
- Equipment for sample disruption and homogenization
- Personal protection equipment (e.g., lab coat, gloves, goggles)

SAFETY INSTRUCTIONS

Always wear chemical resistant gloves, and safety goggles/face-mask/face shield when working with chemicals. Do not inhale or breathe vapor. Do not get into contact with eyes, skin and clothing. Avoid prolonged or repeated exposure. Keep reagents away from heat and open flame. Store in a cool dry place. Wash your hands thoroughly after handling reagents.

Lysis Solution
Contain Tris (hydroxymethyl) aminomethane (Component 1) and Sodium Chloride (Component 2) S 24/25

DNA Binding Buffer
Contain Guanidine Thiocyanate (Component 1) R 20/21/22, R 32, S 22, S 24-25, S 45; Contain beta mercaptoethanol(Component 2): R27,R25,R34,R51S36/37/39, S45

DNA Wash Buffer FWB1
Contain Guanidine Hydrochloride R36, R67, S7, S16, S24/25, S26

R20/21/22: Harmful by inhalation, in contact with skin and if swallowed, R25: Toxic if swallowed, R27: Very toxic in contact with skin, R25: Toxic if swallowed , R32: Contact with acids liberates very toxic gas, R34: Causes burns, R36- Irritating to eyes, R51: Toxic to aquatic organisms, R67- Vapors may cause drowsiness and dizziness, S7- Keep container tightly closed, S16- Keep away from sources of ignition – No smoking , S24/25- Avoid contact with skin and eyes , S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice , S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice, 36/37/39: Wear suitable protective clothing, gloves and eye/face protection, S45: In case of accident or if you feel unwell seek medical advice immediately.

INTRODUCTION

Principle and Procedure

The Nucleopore® DNA Stool kit is designed for the efficient isolation of both microbial and host genomic DNA from fresh and frozen stool samples.

The kit contains a special Lysis Buffer FL1 which, in combination with a 5 minute heating step, leads to a chemical disruption of membranes prior to a mechanical lysis of the sample material using Nucleopore® Beads Tubes Type A (containing ceramic beads) and a mechanical disruption device.

No enzymatic reactions like protease digestion are required to homogenize the sample material.

Undissolved sample material and the ceramic beads are subsequently removed by a short centrifugation. Proteins as well as PCR inhibitors present in the stool sample are precipitated by addition of Lysis Buffer FL2 and a short incubation at refrigerated temperatures, followed by an additional centrifugation step to remove all impurities.

The supernatant is finally cleared by passing it through a Nucleopore® Inhibitor Removal Column that completely removes substances in stool samples that interfere with enzymatic reactions.

Binding conditions are adjusted by addition of Binding Buffer FB to the flowthrough of the Nucleopore® Inhibitor Removal Column and the sample is loaded onto a Nucleopore® DNA Stool Column.

Residual contaminants such as complex polysaccharides, bile salts, and other PCR inhibitors are removed by an efficient washing procedure using Binding Buffer FB and Wash Buffers FWB1 and FWB2. After a drying step, ready to use DNA can be eluted with Elution Buffer FEB.

Specifications of Nucleo-pore Stool DNA Mini Kit

Kit specifications at a glance	
Parameter	Nucleopore ® Stool DNA Mini Kit
Technology	Silica membrane technology
Format	Mini spin column
Sample material	Stool samples (fresh or frozen)
Sample size	180 – 220 mg
Typical yield	2 – 10 µg (varies by sample and disruption device)
Elution volume	30 – 100 µL
Preparation time	60 min/10 preps
Binding capacity	50 µg

Amount of starting material

Nucleopore® DNA Stool is optimized for processing 180 – 220 mg of human stool. For stool samples from animals, lowering the sample amount may lead to better results. Very dry stool samples like rabbit or mouse feces may absorb lysis buffer, resulting in an insufficient sample volume after the first centrifugation step. In these cases it is recommended to reduce the amount of stool material to e.g., 60 – 80 mg and to increase the total lysis volume to 1 mL. A one to one mixture of Buffer FL1 and nuclease free water is recommended for these stool samples.

For difficult stool samples like lipid, polysaccharide, or protein rich stool, a reduction of starting material might also improve the lysis efficiency and the purity of the DNA. It is recommended in such cases to start the extraction with 60 – 80 mg sample material. Human stool samples may also contain undigested food matter (e.g., crop or fruit husks, undigested seeds). These particles should not be transferred to the NP Bead Tubes.

Sample lysis

A thorough sample lysis step is essential to achieve a high DNA yield and remove contaminants during the silica column purification procedure. As stool samples contain a complex mixture of food residues, lipids, proteins, bile salts, and polysaccharides, the chemical lysis by Buffer FL1 is supported by a heating step at 70 °C for 5 minutes. This heating step improves lysis and solubilization of the stool compounds. It is necessary to shake each sample horizontally for 2 – 3 seconds after addition of Buffer FL1 before placing it in the heat incubator in order to mix the stool material and the buffer (take two NP Bead Tubes between thumb and index finger and shake vigorously for 2 – 3 s).

For some animal stool samples, e.g., feces from herbivores like rabbit and sheep, the heat incubation step at 70 °C can be omitted. It is sufficient to perform the bead-beating lysis step with such stool samples only.

The subsequent homogenization step in the NP Bead Tube completely dissolves the stool sample in the lysis buffer and breaks up host and microbial cells. Even solid stool samples such as dried mouse droppings will be suspended after 10 minutes shaking on a Vortex for 30 – 60 seconds at a frequency of 30/s (the best condition for the mechanical lysis on different disruption devices has to be adjusted). Ceramic beads have been proven to be most effective in combination with an NP Bead Tube Holder.

Please refer to the following recommendations to achieve optimal lysis conditions:

Recommended sample input and lysis conditions			
Stool sample	Starting amount	Buffer volumes	Heat incubation
Omnivore and carnivore, e.g., human or feline (Medium to elevated water content, sometimes viscous)	180 – 220 mg	850 µL FL1	Yes
Herbivore, e.g., sheep or rabbit (Medium to low water content, fiber rich)	60 – 80 mg	500 µL FL1 plus 500 µL water	No
Very hard and dry stool, e.g., dried mouse feces (Very low water content)	60 – 70 mg	500 µL FL1 plus 500 µL water	Yes

Lysate clearing and DNA binding

The lysate is cleared in two steps. In the first step, contaminants are precipitated by addition of Lysis Buffer FL2 and incubation at 2 – 8 °C to support the precipitation. In order to achieve an efficient temperature transfer during this short incubation step, it is recommended to use a precooled tube rack on crushed ice.

A Nucleopore® Inhibitor Removal Column is used for the final removal of all residual contaminants from the lysate. After addition of the Binding Buffer FB to the flowthrough of the Nucleopore® Inhibitor Removal Column, the DNA can be bound efficiently to the Nucleopore® DNA Stool Column.

Washing procedure

The washing procedure performed in the Nucleopore® DNA Stool protocol is optimized to remove residual contaminating substances from the DNA bound to the silica membrane. It starts with a washing step with Binding Buffer FB, followed by a second washing step with Wash Buffer FWB1 that also contains guanidinium salt.

The third and fourth washing steps are carried out with Wash Buffer FWB2, which does not contain high salt. The short vortex step in the protocol after the first addition of Buffer FWB2 to the Nucleopore® DNA Stool Column aims to remove all potential guanidinium salt residues from the inside of the column body and the column lid. As guanidinium salt absorbs at 230 nm, this vortex step also helps to improve the A_{260}/A_{230} ratio. The second washing step with Buffer FWB2 can be carried out without prior vortexing of the spin column.

Elution procedures

It is possible to adapt the volume of elution buffer used for the subsequent application of interest. In addition to the standard method, an increase of DNA concentration can be achieved by reducing the elution volume from 100 µL to 30 µL.

If a lower volume than 100 µL is used for elution, it is important to pipette the elution buffer onto the center of the Nucleopore® DNA Stool Column in order to moisten the silica membrane completely.

Incubating the Nucleopore® DNA Stool Column for 1 minute at room temperature after applying the elution buffer may also improve the elution efficiency when using less volume than 100 µL.

If 30 µL are used for elution, the yield can be improved by loading the elution buffer twice onto the spin column. After the first elution step, pipette the 30 µL elution buffer once again from the elution tube onto the membrane of the Nucleopore® DNA Stool Column and centrifuge again for 1 minute at 13,000 x g.

Evaluation of DNA yield and quality

The most common method to determine the DNA yield is UV-VIS spectroscopy. The DNA concentration in the final eluate can be calculated from its absorption maximum at 260 nm (A260). However, this calculation assumes the absence of any other compound that absorbs UV light at 260 nm. Some contaminations significantly contribute to the total absorption at 260 nm and can therefore lead to an overestimation of the actual DNA concentration.

Purity ratio A_{260}/A_{280}

The main indicator of DNA purity is the ratio A_{260}/A_{280}, which should be between 1.7 and 1.9. Values below 1.7 indicate protein contamination.

Purity ratio A_{260}/A_{230}

Another indicator of DNA purity is the ratio of the absorption at 260 nm and 230 nm. A260/
A230 should be higher than 2.0 for pure DNA and can be accepted down to about 1.5.
Ratios around or even below 1.0 indicate impurities in the DNA eluate, which could be of different nature as several compounds absorb at these wavelengths.

Preparation and Storage of Reagents

Attention: Buffers FB and FWB1 contain guanidinium thiocyanate and guanidine hydrochloride, respectively. Wear gloves and goggles!

Storage conditions:

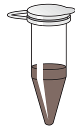
- All kit components should be stored at 15 – 25 °C and are stable until: see package label. Storage at lower temperatures may cause precipitation of salts. If precipitation occurs, incubate the bottle for several minutes at about 30 – 40 °C and mix well until the precipitate is dissolved.

Before starting the first Nucleopore® DNA Stool procedure, prepare the following:

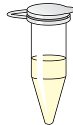
- Wash Buffer FWB2: Add the indicated volume of ethanol (96 – 100%) to Buffer FWB2 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Buffer FWB2 is stable at 15 – 25°C for at least one year.

	Nucleopore ® DNA Stool	
Cat. Nos.	50 preps NP-7011D	250 preps NP-7012D
Buffer FWB2 (Concentrate)	25 mL Add 100 mL ethanol	25 mL Add 100 mL ethanol
		50 mL Add 200 mL ethanol

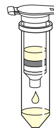
Genomic DNA Purification from Stool



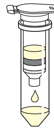
Vortex Stool Sample
With Thrashing Bead
Lysis Tube



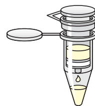
Filter Lysate
With Fast Spin Filter



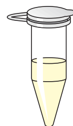
Binding



Washing



Elution



Filter DNA
With Post Elution Filter

Purified DNA

PROTOCOL

Protocol for fresh or frozen stool samples

Before starting the preparation:
- Check Lysis Buffer FL1 for precipitates. Dissolve any precipitate by incubating the buffer at 30 – 40°C for 10 min and shaking the bottle every 2 min.
- Adjust a heating block to 70°C for the initial heat incubation step.

It is recommended to wear lab coat, goggles and gloves throughout the whole procedure

1. Prepare sample

180 – 220 mg sample

+850 µL FL1

Shake horizontally 2 – 3 s

Transfer 180 – 220 mg of human stool material to a **NP Bead Tube Type A.**

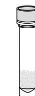
Important: Do not overload the bead tube as this may lead to reduced yield and purity. It is recommended to use an appropriate balance to portion the sample material.

Add **850 µL Buffer FL1.**

Note: For very dry or fiber rich animal stool samples it is recommended to increase the total lysis volume to 1 mL by adding 0.5 mL FL1 Buffer and 0.5 mL nuclease-free water to the sample, as the stool material will take up part of the lysis buffer volume.

Close the NP Bead Tube and shake horizontally for 2 – 3 seconds to mix stool sample and lysis buffer before putting it onto a heat incubator.

2. Lyse sample



70 °C, 5 min

Incubate NP Bead Tubes for 5 min at 70 °C.

Note: For some animal stool samples that contain mostly fibers, e.g., feces from herbivores such as rabbit and sheep, the heat incubation step at 70 °C can be omitted. It is sufficient to perform the bead-beating lysis step

Agitate RT, 10 min

Agitate the Nucleopore® Bead Tube in the NP Bead Tube Holder on a Vortex. Vortex the samples at full speed and room temperature for 10 min.

3. Precipitate contaminants

Centrifuge for **3 min** at **13,000 x *g*.**

Transfer **600 µL** of the supernatant to a fresh **2 mL microcentrifuge tube** with lid (not provided).

Note: If less volume is available, transfer as much lysate as possible to the 2 mL microcentrifuge tube. Avoid transferring material from the pellet or material, which floats on top of the lysate. Fibers or husks in the supernatant may clog the pipette tip. Aspirate the supernatant slowly and carefully.

Add **100 µL Buffer FL2**, close the lid and vortex for 5 s.

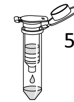
Incubate for 5 min at 2 – 8 °C.

Centrifuge for **3 min** at **13,000 x *g*.**

4. Filter lysate

Place a **Nucleopore® Inhibitor Removal Column** (red ring) in a Collection Tube (2 mL, lid).

Avoiding the pellet, transfer **550 µL** of the **cleared lysate** onto the **Nucleopore® Inhibitor Removal Column.**



Note: If less volume is available, transfer as much cleared lysate as possible to the filter column. Avoid transferring material from the pellet or material, which floats on top of the lysate onto the column.

Centrifuge for **1 min** at **13,000 x *g***.

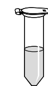
Discard the Nucleopore® Inhibitor Removal Column.

Note: If a pellet is visible in the flowthrough, transfer the clear supernatant to a new 2 mL microcentrifuge tube (not provided).

5. Adjust binding conditions

Add **200 µL Buffer FB** and close the lid.

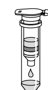
Vortex for 5 s



6. Bind DNA

Place a **Nucleopore® DNA Stool Column** (green ring) in a Collection Tube (2 mL).

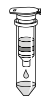
Load 700 µL sample onto the column.



Centrifuge for **1 min** at **13,000 x *g***.

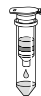
Discard flowthrough and place the column back into thecollection tube.

7. Wash silica membrane



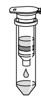
1 st wash

Add 600 µL Buffer FB to the NucleoSpin® DNA Stool Column.
Centrifuge for 1 min at 13,000 x g.
Discard flowthrough and place the column back into the collection tube.



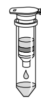
2nd wash

Add 550 µL Buffer FWB1 to the Nucleopore® DNA Stool Column.
Centrifuge for 1 min at 13,000 x g.
Discard flowthrough and place the column back into the collection tube.



3rd wash

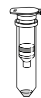
Add 700 µL Buffer FWB2 to the Nucleopore® DNA Stool Column.
Close the lid and vortex for 2 s. Centrifuge for 1 min at 13,000 x g.
Discard flowthrough and place the column back into the collection tube.



4th wash

Add 700 µL Buffer FWB2 to the Nucleopore® DNA Stool Column.
Centrifuge for 1 min at 13,000 x g.
Discard flowthrough and place the column back into the collection tube.
Note: The same collection tube is used throughout the entire washing procedure to reduce plastic waste.

8. Dry silica membrane



Centrifuge for 2 min at 13,000 x g.

Note: If for any reason, the liquid in the collection tube has touched the Nucleopore® DNA Stool Column after the drying step, discard flowthrough and centrifuge again.

9. Elute DNA

Place the Nucleopore® DNA Stool Column into a new 1.5 mL microcentrifuge tube (not provided).

Add 30 µL (for high concentration), 50 µL (for medium concentration and yield), or 100µL (for high yield) Buffer FEB to the column.

Close the lid and centrifuge for 1 min at 13,000 x g.

Discard the Nucleopore® DNA Stool Column.

Vortex each microcentrifuge tube for 2 s.

TROUBLESHOOTING GUIDE

Poor quality DNA

Possible cause

- Buffers and Reagents not re-constituted properly

Suggestions

- Reconstitute buffers solution as per instructions.

Possible cause

- Insufficient cell lysis

Suggestions

- Vortex the mixture vigorously immediately after addition of Lysis Buffer Fl1.

Possible cause

- RNA Contamination

Suggestions

- Add 20ul RNase A solution (20 mg/ml) before addition of Lysis Buffer FL1 if RNA free DNA is required.

Possible cause

- Samples inappropriate.

Suggestions

- Use recommended amount of starting material

Suboptimal performance of genomic DNA in enzymatic reactions

Possible cause

- Ethanol not completely removed

Suggestions

- Make sure to remove all of ethanol before eluting the DNA. If required add a dry spin after DNA Elution at 10,000 x g for 3 minutes

Possible cause

- Co-purification of inhibitory substances

Suggestions

- Use EDTA free elution buffer. It is recommended to use the Elution Buffer FEB provided with the kit.

Low DNA yield

Possible cause

- Inefficient homogenization of samples

Suggestions

- Repeat protocol using new samples and ensure complete homogenization

Possible cause

- Inefficient cell lysis due to insufficient mixing of the sample with Lysis Buffer Fl1

Suggestions

- Repeat the DNA purification procedure with a new sample. Vortex the mixture vigorously immediately after addition of Lysis Solution Fl1.

Possible cause

- Suboptimal elution of DNA from the column

Suggestions

- Preheat Elution Buffer FEB to 70 °C before elution. Apply Elution Buffer FEB directly onto the center of the silica membrane. Check the pH of Elution Buffer FEB as elution efficiency decreases dramatically if elution is performed with buffers of pH < 7.0. It is always recommended to use the Elution Buffer FEB supplied with the kit.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
® DNASure Tissue Mini Kit	50 preps	NP-61305
® DNASure Plant Mini Kit	50 preps	NP-79105
® DNASure Plant Mini Kit	250 preps	NP-79107
® DNASure Plant Midi Kit	20 preps	NP-78153
® DNASure Plant Maxi Kit	10 preps	NP-78164
® DNASure Blood Mini Kit	50 preps	NP-61105
® DNASure Blood Mini Kit	250 preps	NP-61107
® DNASure Blood Midi Kit	20 preps	NP-61184
® DNASure Blood Maxi Kit	10 preps	NP-61193
® DNASure Blood FastPure Kit	50 preps	NP-62205
® DNASure Blood FastPure Kit	250 preps	NP-62207
® SureSpin Plasmid Mini Kit	50 preps	NP-37105
® SureSpin Plasmid Mini Kit	250 preps	NP-37107
® SureSpin Plasmid FastPrep Kit	50 preps	NP-47105
® SureSpin Plasmid FastPrep Kit	250 preps	NP-47107
® SureSpin Buffer Set*	1	37107-BS
® SurePrep Plasmid Mini Kit	20 preps	NP-15123
® SurePrep Plasmid Mini Kit	100 preps	NP-15125
® SurePrep Plasmid Midi Kit	20 preps	NP-15143
® SurePrep Plasmid Midi Kit	100 preps	NP-15145
® SurePrep Plasmid Maxi Kit	10 preps	NP-15161
® SurePrep Plasmid Maxi Kit	25 preps	NP-15162
® SurePrep Plasmid Mega Kit	5 preps	NP-15183
® SurePrep Plasmid Giga Kit	5 preps	NP-15191

***SureSpin® Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin® Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Mini Kit	50	NP-7006D

Product Warranty

Nucleo-pore® Stool DNA Mini kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the Nucleo-pore® Stool DNA Mini kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of Nucleo-pore® Stool DNA Mini kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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