NucleoGene
MDA Detection Kits
Instructions for Use

Release Date— 04.12.2019

REF

For the determination from Fresh or Frozen Human, Animal, Food And Environmental Samples, Water, Microorganism or Microorganism Culture and Swab samples.
For professional use only.

Kit Contents

	Supplied Material	Description	96 or 24 Test
1	Reaction Strips	NucleoGene MDA Reaction Mix	12 or 4 pieces
2	Nucleic Acid Isolation Tubes	Nucleic Acid Isolation Reagent Comprising Tubes	96 or 24 pieces
3	User Manual	Procedure and Process Steps Descriptions	1 piece

Storage Conditions & Durability

NucleoGene Reaction Strips should be stored at -20 ° C.Nucleic acid Isolation Tubes should be stored at + 4 ° C. Do not expose the kit to direct sunlight.The kit can be stored for 12 months without any loss of performance when used under these conditions.

Purpose of Use

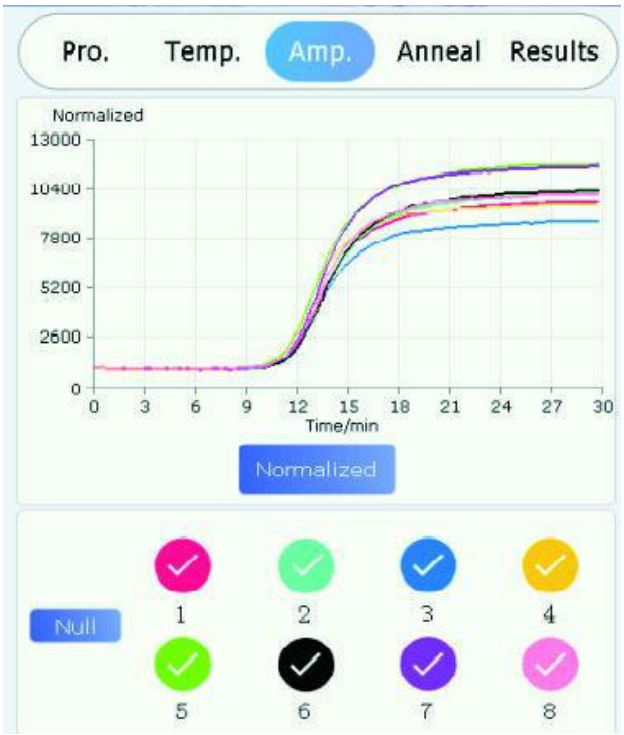
This test was developed to detect microorganism or anythings targets from human, animal, food or environmental samples with simple, fast, high specificity and precision. In the NucleoGene Molecular Detection Assay (MDA) Kit method uses Circular Amplification Technology (CAT METOT), the amplification reaction at a constant temperature proceeds under the isothermal condition. The reaction takes place at high amplification efficiency with a plurality of 10 primers (all other different from techniques) specific to the target gene region without the cycle, so Real Time PCR (hydrolysis probes and hybridization methods) and according to conventional PCR method is completed in less time. The method has high tolerance to inhibitors, thus the human, animal, food and environmental matrix effect is minimized. With its simple applicability, the analysis is completed in 2 steps and in total a maximum of 30 minutes (varies between kits). Amplification of these nucleic acids is carried out by the CAT method using specific gene region-specific primers capable of detecting all target. The presence of microorganism can be easily determined by real-time monitoring of amplification curves in NucleoGene Molecular Detection Assay Instrument. With the specially designed NucleoGene Molecular Detection Instrument or the Real Time PCR, the results do not require electrophoresis or any other method. All steps from amplification to detection are carried out in a reaction tube.

Product Usage Limits

- NucleoGene MDA Detection Kits is intended for the nucleic acid-based diagnosis of human, animal, food and environmental samples. It cannot be used for clinical purposes. It is up to the user's discretion whether the user is suitable for the specific experiment design.
- NucleoGene MDA Detection Kits reaction is very sensitive and contaminated with nucleic acids can cause false results. For this reason, avoid such contamination by performing sample and reagent preparation in different clean benches.
- Rappaport Medium used for selective enrichment of Salmonella spp. should not be used in pre-enrichment since it contains components that affect NucleoGene reaction.
- NucleoGene MDA Detection Kits Do not expose the kit components to UV light. Distortions caused by ultraviolet lamp may lead to incorrect evaluations.
- This kit is intended for the inspection of human, animal, food and environmental samples, not for medical or clinical diagnosis.
- The result of this kit may differ from the culture method.
- The ability of the Nucleic Acid isolation kit to isolate nucleic acid from food and environmental samples in this kit has been confirmed.
- The collection, transportation and storage of the samples to be used are at least as important as purification and are sensitive to the result.
- Designed for professional use only by trained personnel.
- Kits with different lot numbers or any kit components should not be used together.

Amplification Curve Model

Positive Samples



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Warnings and Precautions

- All clinical specimens and residues and debris arising therefrom must be treated as potential infectious agents and disposed of accordingly.
- All samples should be prepared in Biosafety Level 1 or 2 areas or in Class II type Biosafety Cabinets.
- All surfaces, should be freshly prepared bleach with diluted 20% distilled waterand cleaned daily withby using a disposable paper towel or napkin.
- Do not neglect to use laboratory safety devices such as disposable gloves, goggles, visors, disposable cuffs, disposable masks.
- If any of the kit components come into contact with your skin, wash them with plenty of water in no time. In case of contact with your mucus membrane, such as your eyes or mouth, wash the contact area with water again, but do not neglect to consult a physician.
- If possible, prefer pipette tips with filter.
- Keep the kit away from sources of contamination such as DNA and RNA, especially amplified nucleic acid.
- Do not mix solutions with different lot numbers, do not use or combine products from other companies.
- For more information, please refer to the Material Safety Data Sheet (MSDS) which you can request from www.nucleogene.com.

Notes Before You Begin

Before nucleic acid isolation, tubes containing nucleic acid insulating fluids should be mixed with vortex for 10 sec. NucleoGene Molecular Detection Assay (MDA) Kit Reaction Mix Strips The nucleic acid should be used at room temperature after isolation and should be spun with a mini centrifuge before use to ensure that there is no liquid left in the tube walls.

Materials Required(not provided with the Kit)

- Enrichment media (for pre-enrichment)
- Stomacher bag with filter
- Nuclease Free 1.5 ml centrifuge tube
- Micropipette (0.5 ~ 10uL, 10 ~ 100uL, 100 ~ 1,000uL)
- Filtered pipette tips
- Heat block (available at 95 ° C)
- Broken ice and ice box
- NucleoGene Molecular Detection Instrument or Real Time PCR
- Mini centrifuge for strips (optional)
- Vortex mixer

Protocol

1) Pre-Enrichment

In the case of using pre-enrichment culture as an example for the detection of **Salmonella spp**., in foods:

Food 25g+225ml NucleoGene Pre-Enrichment Broth or Buffer Peptone Water (BPW) or EEM Bouillon Media * 2 → Stomach application → (Incubate 37± 1 ° C for 18-24 hours) → pre-enrichment culture

* 2: For pre-enrichment of liver-related materials, NucleoGene Pre-Enrichment Broth or EEM Bouillon Media should be used.

In the case of using pre-enrichment culture as an example for the detection of **Listeria monocytogenes**, in foods:

Food 25g + 225ml NucleoGene Pre-enrichment Broth or Half Fraser media, UVM media, EB media 225mL→Stomach application→ (Incubate 30± 1 ° C for 24 hours) →pre-enrichment culture

In the case of using pre-enrichment culture as an example for the detection of **Escherichia coli O157:H7**, in foods:

Food 25g + 225ml NucleoGene Pre-enrichment Brothor Novobiocin containing mEC media 225mL→Stomach application→ (Incubate 42± 1 ° C for 18-24 hours) →pre-enrichment culture

In the case of using pre-enrichment culture as an example for the detection of **Campylobacter jejuni**, in foods:

Food 25g + 100ml NucleoGene Pre-enrichment Brothor Preston Media→ Stomach application → (Incubate at 42 ° C for 24 hours under microaerophilic condition) →pre-enrichment culture

In the case of using Pre-treatment drink water as an example for the detection of **Legionella pneumophila,** in water:

Put 2mL of sample water (100 times concentrated sample water) in separately prepared 2 ml sterilized tube→13,000-16,000×g, 10 minutes →The tube is 13,000-16,000 × g, 10 minutes is centrifuged.→THA upper liquid is carefully discarded (approximately 1950 microliters), the bacterial cells remain pellet.

* **For other bacteria, use ISO methods or a specific pre-enrichment method that you use in your laboratory.**

2) DNA or RNA Isolation

The pre-enrichment liquid is mixed thoroughly, followed by a 200µL draw with pipette and Nucleic Acid supplied with the kit is transferred to the isolation tube. If swab is used, the nucleic acid is cut into isolation tubes or colonized and suspected colonies are transferred to the Nucleic Acid isolation tubes supplied with the kit.

Remove the scrap from suspected tissue sections for DNA isolation of virus targets and place them in a 1.5 ml tube, then add 100 microliters of nucleic acid isolation fluid. Then follow the procedure below.

- Nucleic Acid Insulation tubes are incubated for 10 minutes at 95 ° C with dry block, shaker heated dry block, water bath or oven and similar equipment.
- At the end of the incubation, the tubes are vortexed for 10 sec and incubated for 5 minutes at room temperature.
- At the end of the incubation, the tubes contain the DNA of the microorganism in the sample used and the DNA is ready to use.
- If the isolated DNA is not used immediately, the tubes should be stored at -20 ° C.

***RNA isolation is done with MTRL (Molecular Transport and Lysis Reagent). It has a procedure that takes 3 minutes in total. Please read and follow the MTRL procedure for viral DNA or RNA isolation.**

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Meat and Food DNA Isolation

Meat and food isolation can be done in two ways in the MDA CAT system. For DNA ISOLATION, the system has been validated in two different isolation kits, NucleoGene One Step DNA Extraction Buffer or NucleoGene Plant and Food Extraction Kit.

NucleoGene One Step DNA Extraction Buffer

1) 20 mg sample is weighed and homogenized thoroughly and transferred to 1.5-2 ml DNase RNase Free microcentrifuge tube.

2) 200 µl of NucleoGene One Step DNA Extraction Buffer is added on it and mixed by vortexing for 30-60 seconds. Then wait 2 minutes at room temperature.

3) The tube is centrifuged for 2 minutes at maximum speed and the supernatant is carefully removed and transferred to a new microcentrifuge tube.

4) The supernatant is diluted 10 times with Nuclease free water and 2 µl is drawn into the reaction tube and the reaction is started.

*For the NucleoGene Plant and Food Extraction Kit, please follow the steps in the kit's instruction manual.

3) NucleoGene MDA Kit Reactions

-NucleoGene MDA Kit Reaction Mix Strip to be used in the reaction is removed from -20 °C to allow it to reach room temperature.

-NucleoGene MDA Kit Reaction Mix Strip when the reactive liquid in the becomes, the strips are placed on ice.

-Than DNA or RNA isolation is completed, containing nucleic acid isolation tubes drawn by taking 2 µL (5 µl for virus kits) DNA or 5 µL RNA, is transferred to NucleoGene MDA Kit Reaction Mix Strip wells.

-Strip is placed in the NucleoGene Molecular Detection Instrument or Real Time PCR. The FAM channel is selected, the device is set to reaction conditions of the kit used.

-When the reaction is complete, the Amplification Curves of the samples are examined first from the result screen.

-Samples with a curve are positive, and samples without a curve are interpreted as negative.

Real Time PCR Protocol

*To run the kits on Real Time PCR device, your device must be compatible with 0.2 ml strip tubes.

-The wells to be used are selected from the Programs section and marked with the FAM or SYBR Green channel.

-The wells are named according to the sample name.

-A single step is selected and the temperature of that kit is entered (for example, Salmonella spp. 65 degrees Celsius).

-The number of cycles is selected as 40 (extending to 60 cycles is optional), each cycle should be set to 30 seconds.

-In each cycle, the device is adjusted to take fluorescence readings and the reaction is started. Results are obtained within 20-30 minutes.

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KITS	REACTION CONDITIONS	REACTION TIME
NucleoGene MDA Salmonella spp. Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Salmonella Enteritidis Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Salmonella Typhimurium Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Salmonella Infantis Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Salmonella Hadar Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Salmonella Virchow Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Salmonella Kentucky Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Salmonella Senftenberg Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Mycoplasma gallisepticum Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Mycoplasma synoviae Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Infectious laryngotracheitis (ILT) Detection Kit (Poultry)	63.5 °C	30 min
NucleoGene MDA Marek's Disease Virus (MDV) Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Group I Avian Adenoviruses Detection Kit (Poultry)	63 °C	30 min
NucleoGene MDA Infectious Bronchitis Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Newcastle Disease Virus Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Avian Influenza A Viruses of subtype H5 Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Avian Influenza A Viruses of subtype H7 Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Avian Influenza A Viruses of subtype H9 Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Avian Metapneumovirus Viruses Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Avibacterium paragallinarum Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Bordetella bronchiseptica Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Ornithobacterium rhinotracheale Detection Kit (Poultry)	65 °C	20 min
NucleoGene MDA Avian Reo Virus (MDV) Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Infectious Bursal Disease Viruses (IBDV) Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Goose parvovirus (GPV) Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Avibacterium paragallinarum Detection Kit (Poultry)	65 °C	30 min
NucleoGene MDA Salmonella spp. Detection Kit (Food)	65 °C	20 min
NucleoGene MDA Listeria monocytogenes Detection Kit (Food)	65 °C	20 min
NucleoGene MDA Escherichia coli O157:H7 Detection Kit (Food)	65 °C	20 min
NucleoGene MDA Campylobacter jejuni Detection Kit (Food)	65 °C	20 min
NucleoGene MDA Legionella pneumophila Detection Kit (Food)	65 °C	20 min
NucleoGene MDA Staphylococcus aureus Detection Kit (Food)	65 °C	20 min
NucleoGene MDA Aeromonas hydrophila Detection Kit (Fish)	65 °C	20 min
NucleoGene MDA Aeromonas salmonicida Detection Kit (Fish)	65 °C	20 min
NucleoGene MDA Aeromonas veronii Detection Kit (Fish)	65 °C	20 min
NucleoGene MDA Aeromonas sobria Detection Kit (Fish)	65 °C	20 min
NucleoGene MDA Infectious Pancreatic Necrosis Virus Detection Kit (Fish)	65 °C	30 min
NucleoGene MDA Infectious Haematopoietic Necrosis Virus Detection Kit (Fish)	65 °C	30 min
NucleoGene MDA Viral Hemorrhagic Septicemia Detection Kit (Fish)	63 °C	30 min
NucleoGene MDA Viral Nervous Necrosis Detection Kit (Fish)	63 °C	30 min
NucleoGene MDA Lactococcus garvieae Detection Kit (Fish)	65 °C	20 min
NucleoGene MDA Photobacterium damselae subsp. piscicida Detection Kit (Fish)	65 °C	20 min
NucleoGene MDA Vibrio alginolyticus Detection Kit (Fish)	63 °C	20 min
NucleoGene MDA Vibrio anguillarum Detection Kit (Fish)	63 °C	20 min
NucleoGene MDA Vibrio harveyi Detection Kit (Fish)	65 °C	20 min
NucleoGene MDA Vibrio vulnificus Detection Kit (Fish)	65 °C	20 min
NucleoGene MDA Yersinia ruckeri Detection Kit (Fish)	63 °C	20 min
NucleoGene MDA Staphylococcus aureus Detection Kit (Food)	65 °C	20 min
NucleoGene MDA Campylobacter spp Detection Kit (Other Animal Disease)	65 °C	20 min
NucleoGene MDA Pasteurella multocida Detection Kit (Other Animal Disease)	65 °C	20 min
NucleoGene MDA Pasteurella spp. Detection Kit (Other Animal Disease)	65 °C	20 min
NucleoGene MDA Pasteurella canis Detection Kit (Other Animal Disease)	65 °C	20 min
NucleoGene MDA Paenibacillus larvae Detection Kit (Other Animal Disease)	65 °C	20 min
NucleoGene MDA Clostridium chauvoei Detection Kit (Other Animal Disease)	65 °C	20 min
NucleoGene MDA Mannheimia haemolytica Detection Kit (Other Animal Disease)	65 °C	20 min

KITS	REACTION CONDITIONS	REACTION TIME
NucleoGene MDA Chlamydia abortus Detection Kit (Other Animal Disease)	65 °C	20 min
NucleoGene MDA Taylorella equigenitalis Detection Kit (Other Animal Disease)	65 °C	20 min
NucleoGene MDA Trypanosoma evansi Detection Kit (Other Animal Disease)	65 °C	30 min
NucleoGene MDA Bovine Viral Diarrhea Virus (BVDV) Detection Kit	65 °C	30 min
NucleoGene MDA Feline infectious peritonitis (FIP) Detection Kit	62 °C	30 min
NucleoGene MDA Leptospira spp. Detection Kit	63 °C	30 min
NucleoGene MDA Brucella spp. Detection Kit	63 °C	30 min
NucleoGene MDA Malaria Detection Kit	65 °C	30 min
NucleoGene MDA Mycobacterium tuberculosis complex Detection Kit	65 °C	30 min
NucleoGene MDA Porcine DNA Detection Kit	65 °C	30 min
NucleoGene MDA Bovine DNA Detection Kit	65 °C	30 min
NucleoGene MDA Chicken DNA Detection Kit	65 °C	30 min
NucleoGene MDA Turkey DNA Detection Kit	65 °C	30 min
NucleoGene MDA Horse DNA Detection Kit	65 °C	30 min
NucleoGene MDA Fish DNA Detection Kit	65 °C	30 min
NucleoGene MDA Sheep DNA Detection Kit	65 °C	30 min
NucleoGene MDA Fish DNA Detection Kit	65 °C	30 min
NucleoGene MDA GMO 35S Detection Kit	65 °C	30 min
NucleoGene MDA GMO NOS Detection Kit	65 °C	30 min
NucleoGene MDA GMO FMW Detection Kit	65 °C	30 min

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