

NucleoGene Molecular Transport and Lysis Reagent (MTLR) Instructions For Use

Published Date — 27.03.2020



For transport and lysis from samples.
Suitable for diagnostic use.
For professional use only.



NG0131-100 ml
NG0131-100 tubes (Spin Column)
NG0131-100tubes (MagneticBeads)

INTRODUCTION

NucleoGene Molecular Transport and Lysis Reagent (MTLR) was developed for the research, epidemiological and surveillance use of the collected samples in remote environments.

NucleoGene MTLR molecular transport medium:

- 1) Obtaining high quality nucleic acids (DNA / RNA) from clinical or environmental samples
- 2) Eliminate potentially infectious biological pathogens for safe transport and handling
- 3) It is a completely different and unique component designed to stabilize the released DNA / RNA for a long time without oxidative / hydrolysis / nuclease degradation. Samples collected in NucleoGene MTLR should be extracted using a commercially available extraction method such as silica spin columns or bead-based extraction systems.

STORAGE

This product is completely ready for use and no further preparation is required. If the product is to be filled into tubes, usually 1.5 ml per sample. The reagent should be used and once dispensed into all liquid tubes in the master stock and stored as such. The product should be transported and stored in its original container at 4-25 ° C until use. NucleoGene MTLR has a 12-month expiration date, clearly labeled on each bottle. Samples collected / stored in NucleoGene MTLR are stable for up to 20 days at ambient temperature (2-30°C). For long periods (> 20 days), samples collected in NucleoGene MTLR should be stored refrigerated or frozen (0 to -80°C).

SPECIMEN

NucleoGene Molecular Transport and Lysis Reagent (MTLR) has been tested and proven in many environmental and animal samples. Acceptable human examples include nasal washes, sputum and nasal / throat / buccal swabs, and other body fluids, including blood. Environmental and cloacal samples were also used and evaluated. These examples may include any bacteria, virus, parasite or something like that. Nucleic acids from samples placed in NucleoGene MTLR are stabilized and maintained at ambient temperature for up to 20 days. NucleoGene MTLR is ideal for clinical, field and application use or high volume sample collection / extraction. Samples collected with NucleoGene MTLR are biologically inactivated and can be sent safely without cooling or dry ice.

Directions for Specimen Collection:

***Before starting the processes, dispense the 1.5 ml NucleoGene MTLR solution into 3 ml tubes with screw cap . If you do not want to do this, ask for NucleoGene MTLR, which is sold distributed to ready-made tubes.**

1. Suitable clinical samples include sputum, nasal wash and throat or nasal swabs.
2. Collect clinical specimens using a flocked swab or other method.
3. After collection, insert flocked swab containing collected sample directly into NucleoGene MTLR collection tube. The volume of sample absorbed onto a flocked swab is typically 0.1 to 0.4 ml. Break-off excess swab handle at the scored mark. For TB sputum collection, swirl a submerged flocked swab 5 times in clinical sputum sample and transfer swab to NucleoGene MTLR tube.
4. Alternatively up to 0.5 ml of clinical material (nasal wash/sputum sample or other bodily fluids including blood) can be pipetted directly into a NucleoGene MTLR. A ratio of 3:1 NucleoGene MTLR to sample is recommended. Place cap back on NucleoGene MTLR and close tightly.
5. Store at room temperature until ready to ship. RNA and DNA from sample collected in NucleoGene MTLR is stable for up to 20 days at ambient temperatures.
6. Proceed with DNA/RNA extraction using a commercially available purification method.
7. Viral RNA from samples collected and stored in NucleoGene MTLR is stable for up to 7 days at ambient temperature (25° C) or can be refrigerated for up to 25 days or frozen.
8. Proceed with RNA/DNA extraction after letting sputum sample sit in NucleoGene MTLR for a minimum of 60 minutes. Extract the RNA/DNA using extraction kits or platforms validated for use with NucleoGene MTLR.

Protocol

Swab Samples

- The sample is taken, then 0.5 ml of MTLR solution is put into a tube and the swab sample is broken into this tube by breaking only the portion of the sample remaining.
- The tube is then capped and shaken vigorously. Meanwhile, waiting (at least 1 hour), nucleic acids of the swab sample (DNA or RNA virus, bacteria, fungus, mold, parasite etc.) are released free of charge. Also, contamination is prevented by making the organism inactive. Nuclease enzymes are inhibited.
- Then, if the spin column method is used, all of the liquid in this tube is transferred to the spin column by skipping the Lysis and Binding stage. Then, it is passed to the washing and elution steps (Wash; 80% ethanol, Elution; Nuclease Free Water). As a result of these steps, nucleic acids are ready for use in a pure manner.
- If magnetic bead isolation method is used, all of the liquid in this tube is transferred to a tube and magnetic beads are added to it and it is waited for



Çavuşoğlu Mahallesi Spor Caddesi,

No:70 Kat:5 Daire:4, 34873

Kartal/Istanbul/TURKEY

E-mail: info@nucleogene.com

Web: www.nucleogene.com

10 minutes at room temperature. Then, it is passed to the washing and elution steps (Wash; 80% ethanol, Elution; Nuclease Free Water). As a result of these steps, nucleic acids are ready for use in a pure manner.

***Apart from these procedures, you can combine the sample with any commercial nucleic acid isolation kit protocol after the MTLR step, depending on the user's desire.**

Liquid and Solid Samples

Liquid; up to 0.5 ml of clinical material (nasal wash/sputum sample or other bodily fluids including blood) can be pipetted directly into a NucleoGene MTLR. A ratio of 3:1 NucleoGene MTLR to sample is recommended. Place cap back on NucleoGene MTLR and close tightly.

Solid; up to 10 mg of clinical material (tissue, soil, food etc.) can be pipetted directly into a 0.5 ml NucleoGene MTLR. Place cap back on NucleoGene MTLR and close tightly. Before this step, the sample needs to be homogenized very well.

- The tube is then capped and shaken vigorously. Meanwhile, waiting (at least 1 hour), nucleic acids liquid or solid samples (DNA or RNA virus, bacteria, fungus, mold, parasite etc.) are released free of charge. Also, contamination is prevented by making the organism inactive. Nuclease enzymes are inhibited.

***Centrifuge(8000xg 10 min) solid samples such as tissue samples after 1 hour of incubation to remove solid particles and use only the liquid portion.**

- Then, if the spin column method is used, all of the liquid in this tube is transferred to the spin column by skipping the Lysis and Binding stage. Then, it is passed to the washing and elution steps (Wash; 80% ethanol, Elution; Nuclease Free Water). As a result of these steps, nucleic acids are ready for use in a pure manner.

- If magnetic bead isolation method is used, all of the liquid in this tube is transferred to a tube and magnetic beads are added to it and it is waited for 10 minutes at room temperature. Then, it is passed to the washing and elution steps (Wash; 80% ethanol, Elution; Nuclease Free Water). As a result of these steps, nucleic acids are ready for use in a pure manner.

***Apart from these procedures, you can combine the sample with any commercial nucleic acid isolation kit protocol after the MTLR step, depending on the user's desire.**

Spin Column

- 1) The MTLR and sample containing tube is transported (or the sample must have waited for 1 hour in the tube).
- 2) After the transport, all the liquid part in the tube is drawn with a pipette and transferred to the spin column placed in the collection tube.
- 3) The tube is centrifuged at 8000 xg for one minute.
- 4) The liquid accumulated in the collection tube is discarded and the spin column is placed back into the collection tube.
- 5) Transfer 500 µl of 80% ethanol to the spin column and centrifuge the tube at 8000 xg for one minute.
- 6) The liquid accumulated in the collection tube is discarded and the spin column is placed back into the collection tube.

- 7) The spin column is centrifuged for one minute at 16000 xg (or at a near maximum speed) so that no residual ethanol remains.
- 8) The spin column is placed in a 1.5 ml microcentrifuge tube.
- 9) 50 µl of Nuclease Free Water is added to the center of the spin column, and it is waited for 1 minute at room temperature.
- 10) The spin column is centrifuged at 8000 xg for one minute. The liquid remaining in the tube contains DNA / RNA. For long term storage, DNA is stored at -20 ° C and RNA at -80 ° C.

Magnetic Beads

- 1) The MTLR(including magnetic beads) and sample containing tube is transported (or the sample must have waited for 1 hour in the tube).
 - 2) After transport, place the tube on the magnetic stand and wait 30 seconds until all magnetic beads have been collected and pipette the discard entire liquid part. Also, remove and discard swab or sample residues from the tube.
 - 3) Take the tube from the magnetic stand, add 500 µl 80% ethanol and mix thoroughly by vortexing or pipette.
 - 4) Put the tube on the magnetic stand and wait 30 seconds until all magnetic beads have been collected and pipette the discard entire liquid part.
- * If desired, Steps 3 and 4 can be repeated.
- 5) Incubate the tubes for 3 minutes at 40 ° C with the caps open to remove any remaining ethanol.
 - 6) Transfer 50 µL of the Nuclease Free Water to the tubes, pre-heated at 70 ° C, vortex or mix well by pipette and incubate for 1-3 minutes at room temperature.
 - 7) After incubation, place the tubes on the magnetic stand and wait for 30 seconds until all magnetic beads are collected and pipette the entire liquid part and transfer it to another tube.
 - 8) The received fluid contains nucleic acid and is ready for use. For long term storage, DNA is stored at -20 ° C and RNA at -80 ° C.

WARNINGS:

DO NOT insert swab into solution before collecting patient specimen.
Do NOT drink, touch or remove NucleoGene MTLR from collection tube.
Do NOT transfer NucleoGene MTLR into other tubes.
Do NOT pool NucleoGene MTLR into larger volumes, or leave tubes uncapped for more than 10 minutes.

First Aid Measures

Skin Contacts; Get medical aid. Immediately flush skin with soap and water while removing contaminated clothing and shoes.

Eye Contact; Immediately flush eyes, occasionally lifting the upper and lower eyelids. Get medical aid immediately.



Inhalation; Remove from exposure to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.

Ingestion; Do not induce vomiting. If victim is conscious and alert, give 2-3 cups of water. Never give anything by mouth to an unconscious person. Get medical aid immediately.



Çavuşoğlu Mahallesi Spor Caddesi,
No:70 Kat:5 Daire:4, 34873
Kartal/İstanbul/TURKEY
E-mail: info@nucleogene.com
Web: www.nucleogene.com