USER'S GUIDE

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MagListo™ cfDNA Extraction Kit

REF K-3618, K-3619

BIONEER
Innovation • Value • Discovery

MagListo™ cfDNA Extraction Kit

Kit for the extraction of cfDNA from plasma, serum, urine, and saliva

User's Guide

K-3618 Σ 4

K-3619 Σ 50

Version No.: 2.0 (2020-09)

Please read all the information in booklet before using the unit

Bioneer Corporation
8-11, Munpyeongseo-ro, Daedeok-gu, Daejeon
34302, Republic of Korea
Tel: +82-42-930-8777
Fax: +82-42-930-8688
Email: sales@bioneer.co.kr
www.bioneer.co.kr

Intended Use

MagListo™ cfDNA Extraction Kit is intended for research use only. This kit is not intended for human or veterinary diagnostics.

Safety Warnings and Precautions

Please inquire Bioneer's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product. *MagListo*™ cfDNA Extraction Kit provides optimized CL buffer, CB buffer and CW1 buffer which are poisonous and hazardous. Please, wear gloves and goggle when working with CL buffer, CB buffer and CW1 buffer.

Before, during and after using this kit, all potentially hazardous materials including tubes, tips and other kit contents should be processed and discarded in accordance with applicable and appropriate regulations of the municipality/government in which this product is being used. A user must also be equipped with basic experimental techniques required for correct execution of the extraction experiments described in this User's Guide.

Warranty and Liability

All Bioneer products undergo extensive Quality Control testing and validation. BIONEER guarantees quality during the warranty period as specified, when following the appropriate protocol as supplied with the product. It is the responsibility of the purchaser to determine the suitability of the product for its particular use. Liability is conditional upon the customer providing full details of the problem to BIONEER within 30 days.

Trademark

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1. Overview

Description

MagListo™ cfDNA Extraction Kit utilizes nano size silica magnetic beads to extract total DNA from a variety of samples, such as plasma, serum, urine, and saliva. The use of *MagListo*™ Magnetic Separation Rack along with the kit greatly increases efficiency and convenience by removing the need of centrifugation.

Features and Benefits

- Magnetic Nano Beads enable rapid nucleic acid extraction
- Both manual and automated extractions are available with *MagListo*™ with *ExiPrep*™96 Lite System
- A single kit serves mini, midi and maxi scale experiments (1 – 5 ml of sample)

Applications

PCR, Real-Time PCR, Digital PCR, Next Generation Sequencing

2. Kit components

MagListo™ cfDNA Extraction Kit Cat. no. K-3618, K-3619	*K-3618 (4 reaction)	*K-3619 (50 reaction)
CL buffer	5 ml x 1 ea	110 ml x 1 ea
CB buffer	5 ml x 1 ea	110 ml x 1 ea
CW1 buffer	5 ml x 1 ea	110 ml x 1 ea
W2 buffer	5 ml x 1 ea	120 ml x 1 ea
EA buffer	1 ml x 1 ea	25 ml x 1 ea
Magnetic nanobead – DNA	1 ml x 1 ea	11 ml x 1 ea
Proteinase K powder, lyophilized	5 mg x 1 ea	25 mg x 2 ea
One page protocol	1 ea	1 ea

3. Storage

MagListo™ cfDNA Extraction Kit should be stored dry at room temperature. It can be stored up to 2 years if properly sealed.

4. Technical Assistance

At Bioneer, we pride ourselves in being responsive to your needs. Our Technical Service Department is staffed with seasoned scientists with extensive expertise in molecular biology. If you have any questions or would like to learn more information about *MagListo*™ products, please contact us. We look forward to hearing from you!

Technical Support

For all technical questions and troubleshooting on Bioneer products and applications.

Tel: +82-42-930-8777

Email: sales@bioneer.co.kr

In North America

Tel: +1-877-264-4300

Email: support@bioneer.us.com

5. Product Specification

	Sample volume		
Plasma / Serum / Urine / Saliva	~ 1 ml	~ 3 ml	~ 5 ml
Elution volume	30 µl	50 µl	70 µl
Typical DNA yield	~ 5 ng	~ 15 ng	~ 25 ng

6. Sample Preparation

Several factors, such as harvesting method and storage of starting samples can influence yield and purity of cfDNA. All specimens must be stored in a freezer or used immediately after collection. It is recommended to process sample on ice as soon as possible, and to avoid multiple cycles of freezing and thawing.

Plasma

Plasma should be collected in a tube containing anticoagulants for blood (EDTA and ACDs). Sample can be stored for few days at 4℃ and at up to 1 year at −70℃. It is recommended that sample be rapidly thawed in water bath (37℃) and then kept on ice before use.

Serum

Serum should be collected in a tube containing a serum separator (SST Tube). Tube can be stored for 7 days at 4℃ and up to 1 year at −70℃.
It is recommended that sample is rapidly thawed in water bath (37℃) and then kept on ice before use.

Urine

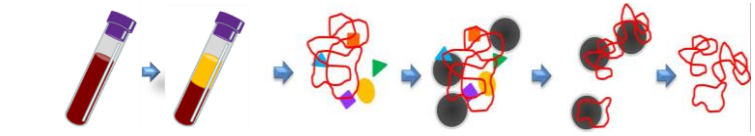
Urine sample should be used immediately or stored in a collection tube. It is recommended to collect sample from first morning urine. Sample can be stored at 4℃ up to 7 days.

Saliva

Saliva sample should be used immediately or stored in a collection tube. Sample can be stored at 4℃ up to 7 days.

7. Principle

MagListo™ cfDNA Extraction Kit is designed for extraction of circulating cell free DNA from plasma, serum, urine and saliva. The overall principle is based on binding of DNA onto Magnetic Nano Beads by chaotrophic salt. Guanidine thiocyanate and hydrochloride act as chaotropic agents in the CL buffer, plus CB buffer and CW1 buffer reduce water content in suspension containing DNA and silica coated magnetic beads, which results in DNA being captured by magnetic beads. The magnetic nanobeads and nucleic acid complexes are pulled and fixed on the tube wall using magnetism, followed by washing with ethanol to remove debris and excessive salts. The captured nucleic acid is then eluted by EA buffer, an aqueous solution with optimal pH.



Whole blood /Urine/Saliva | Plasma / Serum /Urine/Saliva | Lysis | Binding | Washing | Elution

8. Magnetic Nano Bead Information

Description

Silica coated magnetic nanobeads were developed to overcome shortcomings of conventional magnetic beads and to adapt to automation of the purification process.

Features

- Faster binding allows for higher throughput automation
- Large surface area yields greater amount of extraction
- Smooth and globular surface increases purity

Specification

	AccuNanoBead™ Silica Magnetic Nano Beads
Matrix	Silica-coated Fe_3O_4
Average size	400 nm
Ligand	-OH
Working Temp.	0~100℃
Storage	Store at room temperature upon receipt

9. Guidelines for *MagListo*™ Magnetic Separation Rack

Description

MagListo™ Magnetic Separation Rack is designed for a fast, easy separation utilizing magnetism. Bioneer offers racks of different sizes – *MagListo*™ 8Ch for 8-tube strip and multi-pipette, *MagListo*™ 2 for 1.5 Eppendorf tubes or 2 ml tubes, *MagListo*™ 15 for 15 ml tubes, *MagListo*™ 50 for 50 ml tubes.

Recommendations when handling the *MagListo*™ Magnetic Separation Rack

- The rack is made of PMMA and Acryl. Be careful not to drop the product as it may break or cause injury.
- If the product breaks, do not handle with bare hands.
- When an extracted or purified nucleic acid is spilled on the product, immediately rinse it with running water.
- Acetone, toluene, or any organic solvent may damage the PMMA and plastic part of the product, which may result in malfunction. Rinse the product immediately if any solvents are spilled on it.
- Make sure that a corrosive liquid does not spill on the magnet plate part of the product. If spillage occurs, immediately rinse with running water.

10. Materials and Equipment Needed but Not Provided

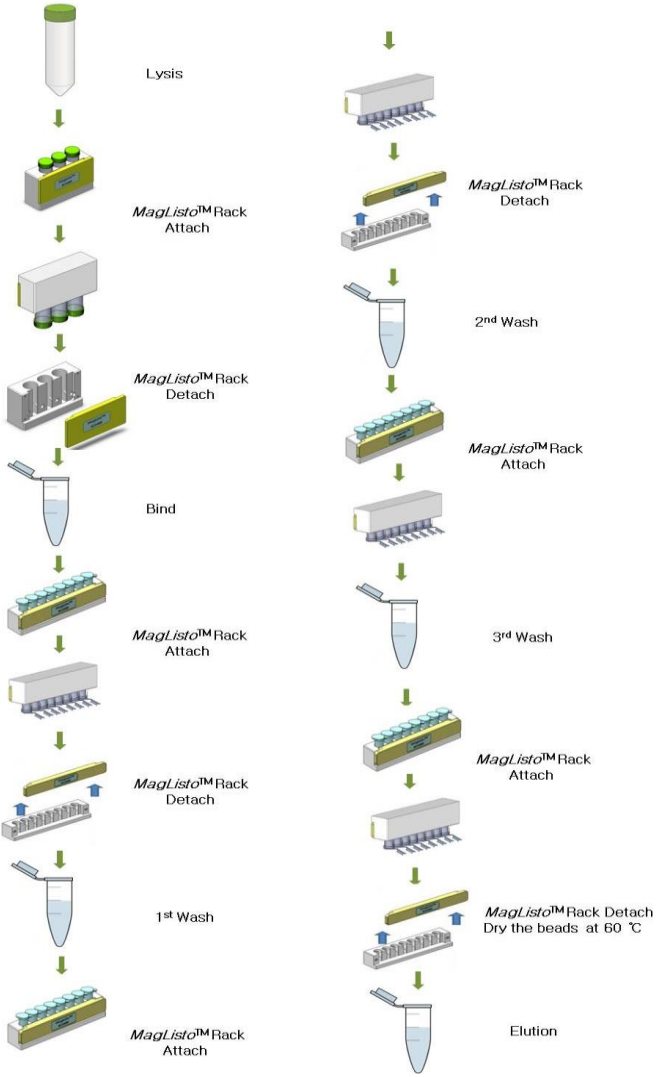
1. 1.5 ml tube, 2 ml tube, 50 ml tube
2. Vortex mixer
3. Centrifuge with rotor capable of 3,000 x *g*
4. Absolute ethanol
5. Thermal block or dry oven
6. *MagListo*™ Magnetic Separation Rack

Types of the Magnetic Separation Rack

Tube	*MagListo*™ Magnetic Separation Rack
1 ml tube with 8-cap strip	*MagListo*™-8Ch Magnetic Separation Rack (Cat. no. TM-1000)
1.5 ml or 2 ml microcentrifuge tube	*MagListo*™-2 Magnetic Separation Rack (Cat. no. TM-1010) *MagListo*™-2-12h Magnetic Separation Rack (Cat. no. TM-1011)
15 ml tube	*MagListo*™-15 Magnetic Separation Rack (Cat. no. TM-1020)
50 ml tube	*MagListo*™-50 Magnetic Separation Rack (Cat. no. TM-1030)

(Note) Please refer to the ordering information in this *User's Guide* for more information regarding catalog number of racks designed for specific size of tubes.

11. Procedure – cfDNA Extraction



12. Protocols

A. Before you begin

1. Completely dissolve Proteinase K (KB-0111) in 1,250 µl of nuclease-free water.
   For short term storage, dissolved Proteinase K should be stored at 4°C.
   For long term storage, it is recommended to aliquot the enzyme into separate tubes and store at -20°C.

2. CL buffer, CB buffer and CW1 buffer contain chaotropic salt. You should take appropriate laboratory safety precautions and wear gloves when handling these substances.

B. Pretreatment of the samples

a. Plasma from whole blood

1. Centrifuge blood at 2,000 x *g* for 10 minutes at 4℃.
2. Transfer supernatant to a 50 ml centrifuge tube.
3. Centrifuge the tube at 2,000 x *g* for 10 minutes at 4℃.
   (Note) The purpose of double spinning plasma is to remove all cellular contaminants and cell debris.
4. Transfer the supernatant to a new 50 ml centrifuge tube.

b. Serum from whole blood

1. Allow the blood samples to clot for 30 minutes to 1 hour at room temperature before centrifugation.
2. Centrifuge at 2,000 x *g* for 10 minutes at 4℃.
3. Transfer supernatant to a new 50 ml centrifuge tube.

c. Urine & Saliva

1. Centrifuge sample at 3,000 x *g* for 30 minutes at 4℃.
2. Transfer supernatant to a new 50 ml centrifuge tube.

C. cfDNA Extraction from Plasma / Serum / Urine / Saliva

1. Transfer the collected sample **(~5 ml)** to a 50 ml tube.

2. Add 40 µl of Proteinase K solution per 1 ml of sample and mix well.

3. (Lysis: 2-4) Add same volume of CL buffer to the collected sample tube.

4. Incubate at 60 ℃ heating block or water bath for 10 min.

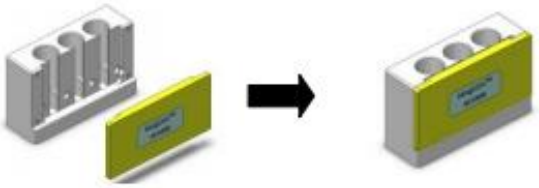
5. (DNA precipitation) Add 375 µl of isopropanol per 1ml of sample to the tube and mix well.

6. (DNA binding with magnetic nano bead) Add 200 µl of Magnetic NanoBeads solution to the tube and mix thoroughly by vortex mixer until the beads are fully resuspended.
   (Note) Magnetic Nano Bead solution contains magnetic nano beads. Please shake well before use.

7. After incubation plate the tube for 5 min in the ice or 10 min at room temperature.

8. Insert the tube in the ***MagListo***™**-50 Magnetic Separation Rack** with the magnet plate attached and invert the rack gently 3 to 4 times until the beads bind tightly to the magnet.

- **Attachment of the magnet plate**

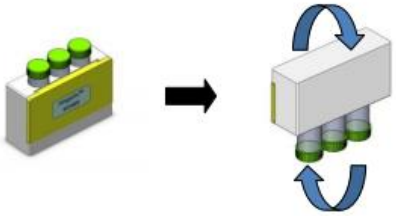


Attach the magnet plate to the stand.

9. Without removing the tubes from *MagListo*™ Magnetic Separation Rack, discard supernatant carefully and completely remove the remaining supernatant onto a paper towel by blotting down.

During this process, the magnetic crude pellet remains attached to the wall of the tube.

– How to discard the supernatant

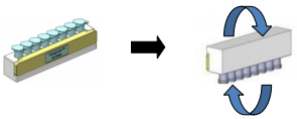


Discard supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling out. When discarding the supernatant, invert the rack completely so that the solution is not spilled on the rack.

10. (1st washing: 10–13) Detach the magnet plate from *MagListo*™ stand. Add 1 ml of CB buffer to the tube. Close the cap and mix by vortex mixer until the beads are fully resuspended.

11. Take the CB buffer mixed with magnetic nano beads and transfer into the new 1.5 ml or 2 ml tube.

12. Place the tube in ***MagListo*™–2 Magnetic Separation Rack** with the magnet plate attached and invert the tube 3~4 times gently until the beads tightly bind to magnet.



13. Without removing the tube from *MagListo*™ rack, discard supernatant carefully and completely remove the remaining supernatant onto a paper towel by blotting down.

14. (2nd washing) Detach the magnet plate from *MagListo*™ stand. Add 1 ml of CW1 buffer, close the cap and mix thoroughly by vortexing. Then repeat the above step 12–13.

15. (3rd washing) Detach the magnet plate from *MagListo*™ stand. Add 1 ml of W2 buffer, close the cap and mix thoroughly by vortexing. Then repeat the above step 12–13.

16. (4th washing) Detach the magnet plate from *MagListo*™ stand. Add 700 µl of absolute ethanol, close the cap and mix thoroughly by vortexing. Then repeat the above step 12–13.

17. (Drying) Open the tubes and completely dry the beads by putting them on a 60℃ thermal block for **15–30 min until residual buffer is completely evaporated.**

18. (Elution: 18–22) Detach the magnet plate from the *MagListo*™ Magnetic Separation Rack. Add **30 µl (1 ml) / 50 µl (3 ml) / 70 µl (5 ml)** of EA buffer or distilled water to each tube and resuspend the bead pellet completely by pipetting or using a vortex mixer for 15 sec.

19. Incubate the tubes at 60℃ for 1 min.

20. Vortex the tubes for 15 sec.

21. Centrifuge at 1,000 rpm for 1 min.

22. Carefully transfer the supernatant containing cfDNA to a sterile tube.

D. Summary of reagents volume in each step

Step	Buffer	Volume			Page
Sample	Plasma / Serum / Urine / Saliva	1 ml	3 ml	5 ml	P. 8
Lysis	Proteinase K	40 µl	120 µl	200 µl	P. 9
	CL buffer	1 ml	3 ml	5 ml	P. 9
DNA Precipitation	Isopropanol	375 µl	1,125 µl	1,875 µl	P. 9
Bead Binding	Magnetic nanobead	200 µl			P. 9
1st Washing	CB buffer	1 ml			P. 10
2nd Washing	CW1 buffer	1 ml			P. 11
3rd Washing	W2 buffer	1 ml			P. 11
4th Washing	Absolute ethanol	700 µl			P.11
Elution	EA buffer	30 µl	50 µl	70 µl	P.11

13. Appendix

A. Troubleshooting guide

This troubleshooting guide will help you to solve problems that may arise during cfDNA extraction. For other technical assistance or more information, please contact our technical assistance team.

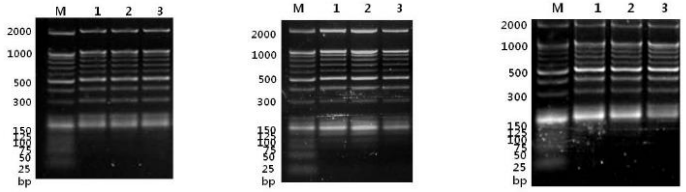
	Comments and suggestions
Low yield of cfDNA	Buffers or other reagents may have been compromised. Please make sure that reagents are stored at room temperature always upon arrival and that all reagent bottles are closed tightly, to preserve pH and stability, and to avoid contamination.
	Elution may have been incomplete. Please extend incubation time up to 3 minutes at the elution step to improve yield. In addition, make sure that magnetic nanobeads are suspended completely in the eluting solution during incubation.
	Too much amount starting sample was used. Appropriate amount of starting sample (see “Kit Specification” in page 4) should be used for efficient extraction of cfDNA.
	Lysis may have been incomplete. Time required for complete lysis varies depending on the type of sample used. A shaking water bath may be used for efficient lysis. Ensure that the starting material is completely disrupted. It may be helpful to increase the incubation time or reduce the amount of starting material.
	A part of magnetic nanobead pellet may have been lost while discarding solution. Check that all nanobeads have bound tightly to the magnet when you discard supernatant
Storage term of cfDNA	Store extracted cfDNA as recommended below: -At 4℃ for up to 24 hours. -At -20℃ for up to long term storage.

Low $A_{260/280}$ ratio	Beads may have been washed insufficiently. You must properly wash the beads in the drying step. Remaining ethanol can decrease purity of DNA. Take enough time to properly wash the beads
	Incomplete suspension of beads during the washing step causes salts to remain in the purified DNA. Make sure that the beads are suspended thoroughly during the washing process.
Magnetic nanobeads not optimally dried	Beads may have dried insufficiently. You must always completely remove the remaining washing solution and dry beads completely in a drying step. Remaining ethanol can be a contaminant to cfDNA. Take plenty of time to completely dry the beads.
Excessively clustered magnetic nanobead	Too much of starting sample is used to extract DNA. Appropriate amount of starting material (see "Kit Specification" in page 4) should be used for efficient extraction of cfDNA.
There is a white precipitate in buffer	A white precipitate may form in CL buffer, CB buffer or CW1 buffer due to prolonged storage at low temperatures. Incubate CL buffer, CB buffer or CW1 buffer at 60℃ to dissolve any precipitate in the buffer.
Degraded DNA	DNA from old or incorrectly stored sample may result in degradation. As yield is highly dependent on storage conditions of sample, please use as fresh sample as possible for best results. In case of using stored sample, it is recommended to store at −70℃.
	Frequent freezing and thawing cycles may result in lower yield than expected. Avoid repeated freezing and thawing.
Extracted DNA floats when loaded on an agarose gel	Floating of DNA on an agarose gel is caused by remaining ethanol in the eluted DNA. Ensure that the drying step in the protocol is properly performed. Remaining ethanol may also interrupt the enzymatic reaction

B. Experimental data

Figure 1. cfDNA size from EDTA human plasma, human urine and human saliva mixed Bioneer ladder purified with *MagListo*™ cfDNA Extraction Kit.

a) Plasma b) Urine c) Saliva

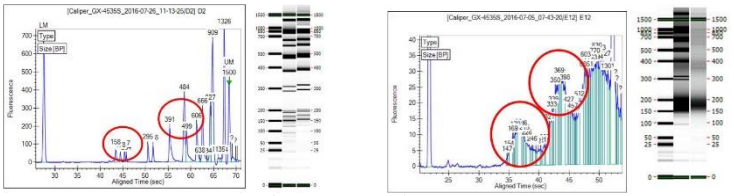


M: Bioneer 25/100bp mixed ladder (Cat. No. D-1020)

a) 1-3: cfDNA of plasma mixed Bioneer ladder purified with Bioneer *MagListo*™ Kit
b) 1-3: cfDNA of urine mixed Bioneer ladder purified with Bioneer *MagListo*™ Kit
c) 1-3: cfDNA of saliva mixed Bioneer ladder purified with Bioneer *MagListo*™ Kit

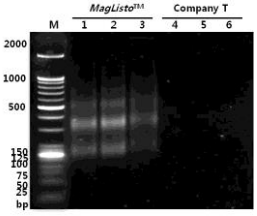
Frigure 2. cfDNA from EDTA human plasma, human serum purified with *MagListo*™ cfDNA Extraction Kit

a) Plasma b) Serum



M: Bioneer 25/100bp mixed ladder (Cat. No. D-1020)
a) cfDNA of plasma mixed Bioneer ladder purified with Bioneer *MagListo*™ Kit
b) cfDNA of serum purified with Bioneer *MagListo*™ Kit

Figure 3. Comparison of cell free DNA from normal human serum (Millipore, 2 ml) purified with *MagListo*™ cfDNA Extraction kit and competitor's kit (magnetic bead type)



M: Bioneer 25 /100 bp mixed ladder (Cat. No. D-1020)
1-3: cell free DNA of serum purified with Bioneer *MagListo*™ Kit
4-6: cell free DNA of serum purified with competitor T kit

14. Ordering Information

Cat no.	Product Description	Size
K-3601	*MagListo*™ 5M Plasmid Extraction Kit, 100 reactions	1 kit
K-3600	*MagListo*™ 5M Plasmid Extraction Kit, 500 reactions	1 kit
K-3603	*MagListo*™ 5M Genomic DNA Extraction Kit, 100 reactions	1 kit
K-3605	*MagListo*™ 5M Plant Genomic DNA Extraction Kit, 100 reactions	1 kit
K-3607	*MagListo*™ 5M Gel Extraction Kit, 100 reactions	1 kit
K-3609	*MagListo*™ 5M PCR Purification Kit, 100 reactions	1 kit
K-3613	*MagListo*™ 5M Universal RNA Extraction Kit, 100 reactions	1 kit
K-3615	*MagListo*™ 5M Forensic Sample DNA Extraction Kit, 100 reactions	1 kit
K-3624	*MagListo*™ 5M Viral DNA / RNA Extraction Kit, 100 reactions	1 kit
K-3623	*MagListo*™ 5M Viral DNA / RNA Extraction Kit, 500 reactions	1 kit
K-3619	*MagListo*™ cfDNA Extraction Kit, 50 reactions	1 kit
TM-1000	*MagListo*™-8Ch Magnetic Separation Rack	1 ml tube x 8 holes
TM-1010	*MagListo*™-2 Magnetic Separation Rack	2 ml tube x 8 holes
TM-1011	*MagListo*™-2-12h Magnetic Separation Rack	2 ml tube x 12 holes
TM-1020	*MagListo*™-15 Magnetic Separation Rack	15 ml tube x 6 holes
TM-1030	*MagListo*™-50 Magnetic Separation Rack	50 ml tube x 3 holes
HT-15-NG	Graduated 1.5 ml Microcentrifuge Tubes	500 ea / pk

15. Explanation symbols

REF	Catalog Number		Contains sufficient for (n) tests		USE BY
LOT	Batch code		Caution, consult accompanying documents		Temperature Limitation
	Manufacturer		Caution, Potential Biohazard		Consult Instruction For Use

Bioneer Worldwide

Bioneer Corporation

Address	8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302, Republic of Korea
Tel	+82-42-930-8777 (Korea: 1588-9788)
Fax	+82-42-930-8688
E-mail	sales@bioneer.com
Web	www.bioneer.com

Bioneer Inc.

Address	155 Filbert St. Suite 216 Oakland, CA 94607, USA
Tel	+1-877-264-4300 (Toll-free)
Fax	+1-510-865-0350
E-mail	order.usa@bioneer.com
Web	us.bioneer.com

Bioneer R&D Center

Address	Korea Bio Park BLDG #B-702, 700 Daewangpangyo-ro, Bundang-gu, Seongnam-si Gyeonggi-do, 13488, Republic of Korea
Tel	+82-31-628-0500
Fax	+82-31-628-0555
E-mail	sales@bioneer.co.kr
Web	www.bioneer.co.kr