USER GUIDE

MagListo™ 5M Universal RNA Extraction Kit

Cat. No. K-3613

BIONEER
Innovation • Value • Discovery

MagListo™ 5M Universal RNA Extraction Kit

Kit for the extraction of RNA from cultured cells, plant tissues, or animal tissues

User Guide

K-3613

Σ 100

Version No.: 4 (2022-05-25)

Please read all the information in booklet before using the unit

BIONEER Corporation
Bioneer Global Center, 71, Techno-2-ro,
Yuseong-gu, Daejeon, 34013, Republic of Korea
Tel: 1588-9788
Email: sales@bioneer.co.kr
www.bioneer.com

Intended Use

MagListo™ 5M Universal RNA Extraction Kit is developed and supplied for research purposes only. Certain applications possible with this kit may require special approval by appropriate local and/or national regulatory authorities in the country of use.

Safety Warning and Precaution

Wear appropriate protection when handling any irritant or harmful reagents. The use of a laboratory coat, protective gloves and safety goggles are highly recommended. For more information, please consult the appropriate Material Safety Data Sheet (MSDS).

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.

Quality Management System ISO 9001 Certified

Every aspect of our quality management system from product development, production to quality assurance and supplier qualification meets the world-class standards.

Patent

MagListo™ and its kits are protected by the patents KR10-2015-0089172.

Trademark

MagListo™ is a trademark of BIONEER Corporation.

Copyright

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Contents

Product Information

Components

Components	Amount*	Storage
Magnetic Nano Bead	11 ml x 1 ea	Store at room temperature (15-25°C).
RD Buffer (RNA Binding)	30 ml x 2 ea	
RWM1 Buffer (1st Washing)	90 ml x 1 ea	
RWA2 Buffer (2nd Washing)	100 ml x 1 ea	
WE Buffer (3rd Washing)	120 ml x 1 ea	
ER Buffer (Elution)	20 ml x 1 ea	
One Page Protocol	1 ea	

*** Note:** Mini – 100 rxn, Midi – 10 rxn

Storage

The kit will maintain performance for at least two years under standard storage conditions.

Specifications

***MagListo*™ 5M Universal RNA Extraction kit**			
Amount of Starting Sample	Cultured cells		10^4-10^8 cells
	Liver		25-50 mg
	Spleen		100 mg
	Plant Tissues		100 mg
Typical RNA Yield	Cultured cells		15-20 µg
	Liver		10-60 µg
	Spleen		30-60 µg
	Plant Tissues		70-80 µg
Typical RNA Yield	Scale	Mini	up to 100 µg
		Midi	up to 500 µg
Turnaround Time		Mini	< 10 min
		Midi	< 15 min
Elution Volume		Mini	50 µl
		Midi	500 µl
RNA Purity			A_{260}/A_{280} > 2.0, A_{260}/A_{230} > 1.7
Isolation Technology			Magnetic Nano Bead

*** Note:** There may be differences in measured values depending on the type of samples.

Precautions

- Take appropriate laboratory safety precautions and wear gloves when handling because RD Buffer contains chaotropic salts which are irritants.
- RNA is highly susceptible to degradation by exogenous RNase that may be introduced during the handling steps, all the steps must be conducted under sterile, RNase-free condition.
- RNase-free reagents, pipette tips, and tubes must be used with gloved hands while handling them.

Introduction

Product Description

MagListo™ 5M Universal RNA Extraction Kit is designed for extraction of highly purified RNA from cultured cells, plant and animal tissues. The kit employs Magnetic Nano Beads to extract total RNA with the aid of *MagListo*™ Magnetic Separation Rack and *ExiPrep*™ 96 Lite (Cat. No. A-5250). The use of *MagListo*™ Magnetic Separation Rack along with the kit greatly increases user convenience by shortening the extraction time without centrifugation. On the same principle, *ExiPrep*™ 96 Lite is designed for rapid extraction of nucleic acids delivering up to 96 extracted samples (including controls).

RNA extracted through this kit can be used for a variety of applications, including: reverse transcription PCR (RT-PCR), reverse transcription quantitative PCR (RT-qPCR), northern blot analysis, and cDNA synthesis.

Principle

MagListo™ 5M Universal RNA Extraction Kit uses Magnetic Nano Beads to extract nucleic acid. Buffers within the kit assist nucleic acid to bind to silica-coated magnetic nanobeads. As a result, high yield and highly purified nucleic acid is extracted from samples.

The kit consists of lysis & binding buffer, washing buffer, elution buffer, and magnetic nanobeads. Samples are lysed and homogenized in the presence of a guanidine-thiocyanate-containing buffer, which is a highly denaturing agent and inactivates RNase to isolate RNA. Extracted RNA is bound to silica-coated magnetic nanobeads. Cell debris and other contaminants are eliminated by subsequent washing, and highly purified RNA is eluted in an elution buffer or RNase-free water.

*** Note:** Some samples require pre-treatment step.

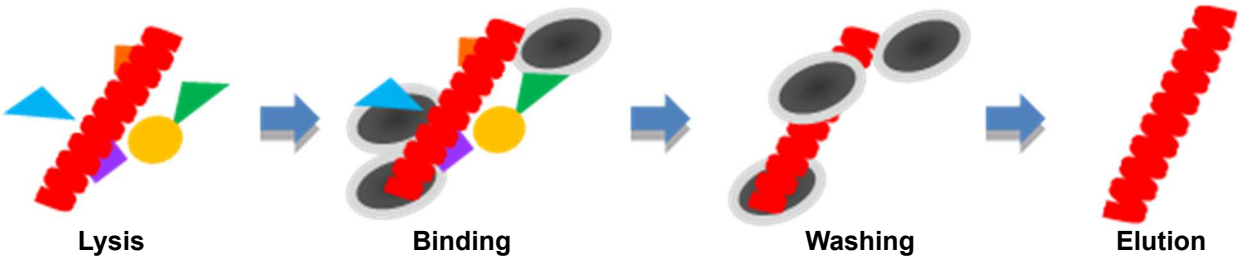

Figure 1. RNA extraction using silica-coated magnetic nanobeads.

Features & Benefits

- Comprehensive: High quality and yield of RNA extraction from various samples such as plant tissues, animal tissues, or cultured cells.
- Convenient: Broad coverage of scales for mini and midi isolation protocols with just a single kit (Mini-10 min, Midi-15 min).
- Rapid: Magnetic Nano Beads enable the rapid nucleic acid extraction.
- Cost-effective: Can be applied to *ExiPrep*™ 96 Lite to automate RNA extraction.

Magnetic Nano Beads

Magnetic Nano Beads have been developed to overcome defects of existing resin and to automate purification process. The principle of extraction using the magnetic nanobeads is that coated functional group on the surface of it binds to nucleic acid. Magnetic nanobeads are then isolated using external magnetic field.

Specification

Silica-coated Magnetic Nano Beads	
Matrix	Silica-coated Fe_3O_4
Average size	200 nm
Ligand	- OH
Working Temperature	0-100°C
Storage	Store at room temperature (15-25°C).

Features & Benefits

- Rapid: Fast binding guarantees high throughput automation.
- Effectiveness: Large surface area enables more sensitive assay.
- Specificity: Globular structure increases specificity by decreasing non-specific binding.

MagListo™ Magnetic Separation Rack

MagListo™ Magnetic Separation Rack is designed for a fast and easy separation of the Magnetic Nano Beads. BIONEER offers various racks of different sizes - *MagListo*™-8Ch for 8-tube strip and muti-pipette, *MagListo*™-2 for 2 ml tube, *MagListo*™-15 for 15 ml tube, and *MagListo*™-50 for 50 ml tube. These racks consisting of different size allow user to choose the product according to their needs.

The followings are recommended when handling the *MagListo*™ Magnetic Separation Rack.

- The product is made of acryl and plastic. Be careful not to drop the product as the dropping may break the product.
- When moving the product, take extra care not to drop the product as it may cause injury.
- If the product is broken, do not discard it with bare hands as the sharp edges may cause injury.
- When an extracted or purified nucleic acid is spilled on the product, immediately rinse it with running water and clean it with 70% ethanol.
- Acetone, toluene, or organic solvent may cause damage to the acrylic and plastic part of the product, which may lead to malfunction of the product. Rinse the product immediately when the above-mentioned solvent leaks as the expected DNA yield may not be obtained if the product is damaged.
- Check the magnet plate part of the product for corrosive liquid. In the event of a sill, it may corrode the magnet during storage and may deteriorate performance, so rinse immediately with running water.

Features & Benefits

- Rapid: Isolates plasmid DNA, genomic DNA, and/or RNA quickly (within 20 minutes) and economically.
- Convenient: Simply disposes waste solution just by flipping the rack without centrifugation or pipetting as the silicon fixture secures the tube.

Experimental Procedures

Before You Begin

Before proceeding, please check the following:

1. Add 10 µl of β-mercaptoethanol per 1 ml of RD Buffer.

2. g-force can be calculated as follows: rcf = 1.12 x r x $(rpm/1,000)^2$

   *** Note:** Where 'rcf' is the relative centrifugal force (in g), 'r' is the radius of the rotor in centimeters, and rpm is the speed of the centrifuge in revolutions per minute.

Preparing Lysates from Cultured Cells

1. **(Cell harvesting)** Harvest cells according to step 1-A or 1-B.

1-A. Suspension cell culture:

Harvest cultured cells (10^4-10^6 cells, mini)* by centrifugation at 300 x *g* for 5 minutes to pellet cells. Discard the supernatant carefully without disturbing a cell pellet and go to step 2.

*** Note:** The amount of sample required may vary depending on the extraction scale.

1-B. Monolayer cell culture:

There are two different methods to collect cells grown in monolayer culture.

a. Direct cell harvesting on the culture dishes:
   Completely discard the cell culture medium and go to step 2.
   *** Note**: You should completely remove the cell culture medium because it may inhibit the RNA extraction.

b. Cell harvesting with trypsin:
   Remove the cell culture medium and wash the cell monolayer with DPBS. Add 0.1-0.25% trypsin to the washed cell monolayer to detach the cells. After the cells detach, add cell culture medium. Transfer the cells to an RNase-free tube (not provided) and centrifuge at 300 x *g* for 5 minutes. Discard the supernatant carefully and go to step 2.

2. **(Lysis & homogenization)** Resuspend the cell pellet from step 1 in 500 µl (mini) / 5 ml (midi) of RD Buffer by vortexing.
   *** Note:** You should completely resuspend the sample to achieve maximum lysis efficiency.

3. **(RNA precipitation)** Add 300 µl (mini) / 3 ml (midi) of absolute ethanol† (not provided) to the lysate and mix immediately by pipetting.
   *** Note:** Do not centrifuge.
   † When purifying RNA from certain cell lines, precipitates may be visible after addition of ethanol. This does not affect the procedure.

4. Proceed immediately to “Purification Procedure Using Magnetic Nano Beads” on page 12.

Preparing Lysates from Plant Tissue

1. **(Sample preparation)** Grind ≤ 100 mg of plant sample (mini)* in liquid nitrogen† to a fine powder with a mortar and pestle and place them into an appropriately sized tube.
   * **Note:** The amount of sample required may vary depending on the extraction scale.
   * **Note:** Do not allow the sample to thaw.
   † After grinding, liquid nitrogen should be evaporated.

2. **(Lysis & homogenization)** Add 500 µl / 5 ml (midi) of RD Buffer to the sample and mix thoroughly by vortexing.

3. Incubate at 60°C for 1-3 minutes. A short 1-3 minutes incubation at 60°C may help to disrupt the tissue. Centrifuge at full speed for 2 minutes and transfer the supernatant to an appropriately sized tube.

4. **(RNA precipitation)** Add 300 µl / 3 ml (midi) of absolute ethanol (not provided) to the lysate and mix immediately by pipetting.
   * **Note:** Do not centrifuge.

5. Proceed immediately to “Purification Procedure Using Magnetic Nano Beads” on page 12.

Preparing Lysates from Animal Tissue

1. **(Lysis & homogenization)** Grind (or homogenize) 20-30 mg (mini)* of fresh or thawed animal tissue sample with a mortar and pestle (or homogenizer) and place it into appropriate tubes.

   *** Note:** The amount of sample required may vary depending on the extraction scale.

2. Add 500 µl (mini) / 5 ml (midi) of RD Buffer to the sample and mix thoroughly by vortexing.

3. Centrifuge at full speed for 3 minutes and carefully transfer the supernatant to a new 1.5 ml tubes (mini) or 50 ml tubes (midi) with a pipette.

4. **(RNA precipitation)** Add 300 µl (mini) / 3 ml (midi) of absolute ethanol (not provided) to the lysate and mix immediately by pipetting.

   *** Note:** Do not centrifuge.

5. Proceed immediately to “Purification Procedure Using Magnetic Nano Beads” on page 12.

Purification Procedure Using Magnetic Nano Beads

1. **(RNA binding)** Add 100 µl (mini) / 1 ml (midi) of Magnetic Nano Bead solution to each tube and mix thoroughly by vortexing until the beads are fully resuspended.

2. Place the tube in *MagListo*™-2 (mini) or *MagListo*™-50 (midi) Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

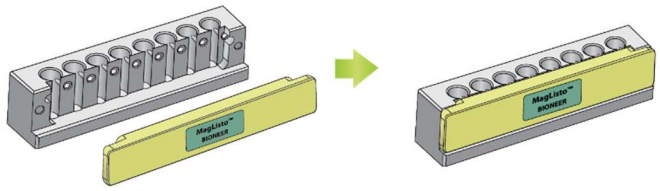

Figure 2. Attachment of the magnet plate. Combine the magnet plate to the stand.

3. Without removing the tube from *MagListo*™ Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting. During this process, the beads containing RNA remain attached to the side of the tube.

   *** Note:** If you want to perform the optional RNA Clean-Up, follow the steps on page 15 after performing this step.

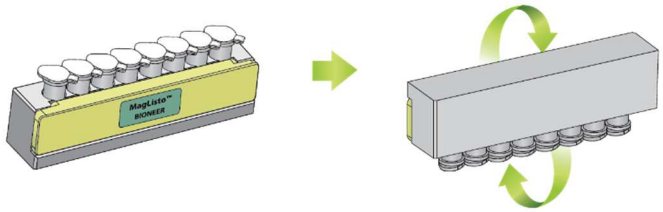

Figure 3. How to discard the supernatant. Discard the supernatant by inverting the *MagListo*™ Magnetic Separation Rack. The silicone immobilizer inside the stand prevents the tubes from falling. When discarding the supernatant, invert the rack completely so that the solution does not spill on the rack.

4. **(1st Washing)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 800 µl (mini) or 8 ml (midi) of RWM1 Buffer to each tube and close the cap. Mix by vortexing until the beads are fully resuspended.

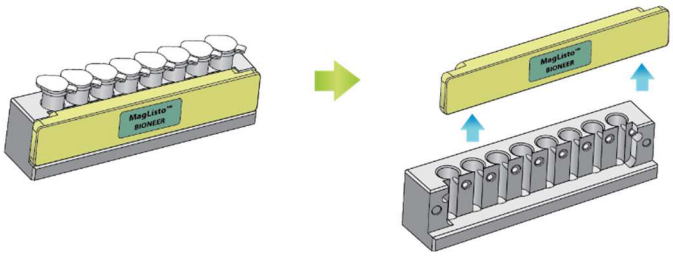

Figure 4. Detachment of the magnet plate. Pull the magnet plate up and gently separate it.

5. Attach the magnet plate to stand and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

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

7. **(2nd Washing)** Repeat steps 4-6 by adding 800 µl (mini) or 8 ml (midi) of RWA2 Buffer for additional washing. Repeat steps 5-6 once more.

8. **(3rd Washing)** Remove residual ethanol according to step 8-A or 8-B.

8-A. Washing beads:

Without removing the tube from *MagListo*™ Magnetic Separation Rack, add 700 µl (mini) or 10 ml (midi) of WE Buffer to the opposite side of beads. Close the cap and invert the rack twice in order to remove ethanol from the sample. Discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

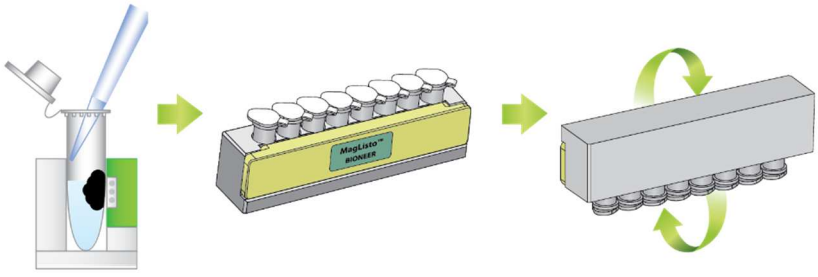

Figure 5. Washing the beads to remove residual ethanol. Please refer to the information above.

*** Note:** Direct pipetting of WE Buffer onto the beads, vortexing and/or vigorous shaking of the tubes may

release nucleic acid from the beads, which may result in lower RNA yield.

8-B. Drying beads:
Add 800 µl of 80% ethanol, mix thoroughly by vortexing, and repeat the steps 5-6. Completely dry the beads with the tube open at 60°C for at least 5 minutes. Remove the remaining supernatant with a pipette.

9. **(Elution)** Detach the magnet plate from *MagListo*™ Magnetic Separation Rack. Add 50-100 µl (mini) or 500 µl-1 ml (midi) of ER Buffer to each tube and resuspend RNA by vortexing or pipetting.

10. Incubate at 55-65°C for 1 minute.

11. Attach the magnet plate to *MagListo*™ Magnetic Separation Rack and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

12. Without removing the tube from *MagListo*™ Magnetic Separation Rack, transfer supernatant containing RNA carefully to a new tube.

13. Discard the tubes with the remaining beads.
*** Note:** Do not reuse the beads.

RNA Clean-Up

1. Adjust the sample to a volume of 100 µl with RNase-free water.
   * **Note:** If DNA-free RNA is required, add RNase-free DNase and DNase reaction buffer to each tube and adjust the volume up to 100 µl with RNase-free water. Incubate at room temperature for 10 minutes.

2. Add 100 µl of RD Buffer and mix well by pipetting.

3. Add 200 µl of absolute ethanol and mix well by pipetting.

4. Add 100 µl of Magnetic Nano Beads and mix well by pipetting until the beads are fully resuspended.
   * **Note:** Please mix well Magnetic Nano Beads by vortexing before use.

5. Place the tube in *MagListo*™-2 (mini) Magnetic Separation Rack with the magnet plate attached and invert the rack gently 3-4 times until the beads bind tightly to the magnet.

6. Without removing the tube from *MagListo*™ Magnetic Separation Rack, discard the supernatant carefully and completely remove the remaining supernatant using a paper towel by blotting.

7. Go to step 4 of “Purification Procedure Using Magnetic Nano Beads” on page 12.

Troubleshooting

Problem	Comments
Low RNA yield	• **Buffers or other reagents may have been exposed to conditions that reduce their effectiveness.** Store the reagents at room temperature (15-25°C) at all times upon arrival and keep all reagent bottles tightly closed after use to preserve pH and stability and avoid contamination.
	• **Excess amount of starting sample may have been used for RNA extraction.** Appropriate amount of starting sample should be used for efficient RNA extraction.
	• **Elution may have been incomplete.** Please expand the incubation time up to 3 minutes at elution step. In addition, make sure that Magnetic Nano Beads are suspended completely in the eluting solution during incubation.
	• **Insufficient shaking or vortexing during lysis step may lead to low RNA yield.** Shake or mix thoroughly by vortexing during lysis step.
	• **Cell culture medium may have been incomplete.** Remove the cell culture medium as much as possible. Any leftover in the medium can lead to an inhibition of RNA extraction
Low RNA purity	• **Magnetic Nano Beads may have been washed insufficiently.** Properly wash the magnetic nanobeads in the 3rd washing step. Remaining ethanol can decrease the RNA purity. Take enough time to wash properly.
Degraded RNA	• **There may have been RNase contamination.** Use a heat gun or blow dryer in a clean bench to prevent the RNase contamination in the air. Use RNase-free pipette tips and change the gloves frequently.
	• **The sample may have been stored inappropriately.**

	Cultured cell samples and lysed samples with RD Buffer should be stored at -80°C.
	• **Repeated freezing and thawing may degrade RNA.** Avoid repeated freezing and thawing.
Sample floating upon loading in an agarose gel	• **Sample may contain ethanol.** Floating is caused by remaining ethanol. Ensure that the 3rd washing (ethanol removing) step in the protocol is properly performed.

Appendix A

Long term storage of RNA in formamide

1. Dissolve RNA pellet in deionized formamide.

2. Add NaCl to the final concentration of 0.2 M followed by 4 volumes of ethanol to precipitate RNA from formamide.

3. Incubate at room temperature for 10 minutes.

4. Centrifuge at 12,000 rpm for 5 minutes at room temperature.

Appendix B

Measurement of absorbance of RNA samples

The A_{260}/A_{280} ratio is a commonly used criterion for nucleic acid purity. Values for pure RNA are usually >1.8. However, the absorbance of nucleic acids at these wavelengths is dependent upon the ionic strength and pH of the medium. The change in the A_{260}/A_{280} ratio is primarily due to the decrease in the absorbance at 280 nm when the ionic strength or pH is increased. We recommend that diluting the RNA with a TE buffer for spectrophotometric assays.

1. Measure the volume of the total RNA sample.

2. Transfer 1 µl of total RNA sample to a 1.5 ml tube.

3. Add 999 µl of TE (pH 8.0) buffer and mix by pipetting.

4. Measure A_{260} and A_{280} with TE (pH 8.0) buffer as a reference blank.

5. Calculate RNA yield as follows:
   1 A_{260} unit of RNA = 40 µg/µl
   Total A_{260} = (A_{260} of diluted sample) x (dilution factor)
   Concentration (µg/ml) = (total A_{260}) x (40 µg/µl)
   Yield (µg) = (total sample volume) x (concentration)

6. Calculate the A_{260}/A_{280} ratio. Pure RNA exhibits A_{260}/A_{280} > 2.0.

References

Bonham, M. J., & Danielpour, D. (1996). Improved purification and yields of RNA by RNeasy®. *Biotechniques*, *21*(1), 57-60.

Coombs, N.J., Gough, A.C., and Primrose J.N. (1999) Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. *Nucleic Acids Research*, *27*(16), e12.

Reno, C., Marchuk, L., Sciore, P., Frank, C.B., and Hart, D.A. (1997) Rapid isolation of total RNA from small samples of hypocellular, dense connective tissues. *Biotechniques*, *22*(6), 1082-1086.

Ordering Information

Description	Cat. No
MagListo™ 5M Universal RNA Extraction Kit	K-3613

Related Products

Description	Cat. No
MagListo™-8Ch Magnetic Separation Rack	TM-1000
MagListo™-2 Magnetic Separation Rack	TM-1010
MagListo™-2-12h Magnetic Separation Rack	TM-1011
MagListo™-15 Magnetic Separation Rack	TM-1020
MagListo™-50 Magnetic Separation Rack	TM-1030

Explanation of Symbols

LOT Batch Code	Consult Instructions For Use	RUO Research Use Only	Caution
Biological Risks	Contains Sufficient for <n> tests	Temperature Limitation	Manufacturer
REF Catalog Number	Do not Re-use	Use-by Date	

BIONEER Corporation - HQ

Address 8-11 Munpyeongseo-ro, Daedeok-gu, Daejeon, 34302, Republic of Korea
E-mail sales@bioneer.co.kr
Web www.bioneer.com

BIONEER Global Center

Address 71, Techno 2-ro, Yuseong-gu, Daejeon, 34013, Republic of Korea
E-mail sales@bioneer.co.kr
Web www.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
E-mail sales@bioneer.co.kr
Web www.bioneer.com

BIONEER Inc. - USA Branch

Address 155 Filbert St. Suite 216 Oakland, CA 94607, USA
E-mail order.usa@bioneer.com
Web us.bioneer.com

BIONEER Corp. - European Branch

Address Ludwig-Erhard-Strasse 30-34, 65760 Eschborn, Germany
E-mail euinfo@bioneer.com
Web www.bioneer.com

BIONEER
Innovation • Value • Discovery