

ddSEQ™ Single-Cell Isolator

Instruction Manual

Catalog #12004336



BIO-RAD

Bio-Rad Technical Support

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Safety and Regulatory Compliance

This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2010 (3rd ed.), EN 61010-1:2010 (3rd ed.). Electrical Equipment for Measurement, Control, and Laboratory Use — Part 1: General requirements
- EN 61326-1:2006 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General requirements
- UL 61010-1:2004, Laboratory equipment, Test & Measurement Equipment and Industrial Process Controls
- CAN/CSA 22.2 No 61010-1-04, Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part I: General Requirements

This equipment generates, uses, and can radiate radiofrequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense.

This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.



The CE mark indicates that the manufacturer ensures the product conforms with the essential requirements of the applicable EN directives.



The CSA mark indicates that a product has been tested to Canadian and U.S. standards, and it meets the requirements of those applicable standards.



The Waste Electrical and Electronic Equipment Directive symbol indicates that when the end-user wishes to discard this product, it must be sent to separate collection facilities for recovery and recycling.

Instrument Safety Warnings

Alteration of this instrument voids the warranty and safety certification and creates a potential safety hazard. This instrument is intended for laboratory use only. Bio-Rad Laboratories is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended, or by modifications to the instrument not performed by Bio-Rad Laboratories or an authorized agent. Follow the safety specifications listed here and throughout this manual. Use only the power cord supplied with the instrument, using only the plug adaptor that corresponds to the electrical outlets in your region. Use of unapproved reagents may harm the instrument and voids the warranty.

Biological and Chemical Hazards

The ddSEQ Single-Cell Isolator is a laboratory product. If operation involves the use of samples that are biological or chemical hazards, adhere to the following necessary safety precautions and comply with any local, state/provincial, and/or national regulations specific to your laboratory and location.

- Handle all infectious samples according to good laboratory practices and methods to prevent the spread of disease. This includes wearing proper personal protective equipment (PPE) and using the proper level of containment required. Refer to your institution's Environmental Health & Safety guidelines for samples at different biosafety levels
- Observe proper safety precautions for aerosol containment. Improper use of the instrument may generate aerosols. Reduce the risk of aerosol generation by ensuring all components are added to cartridges prior to starting run
- Dispose of all waste solutions according to appropriate environmental health and safety guidelines

Table of Contents

Chapter 1. ddSEQ Single-Cell Isolator	1
1.1 Introduction	1
1.2 ddSEQ Single-Cell Isolator	2
1.3 Installation	4
1.4 Instrument Test	5
Chapter 2. Single-Cell Isolation	7
2.1 Best Practices for Loading the Cartridge	7
2.2 Operation of the ddSEQ Single-Cell Isolator	9
2.3 Best Practices for Droplet Transfer	10
Chapter 3. Specifications and Maintenance	11
3.1 Specifications	11
3.2 Maintenance	12
Ordering Information	13

1

ddSEQ Single-Cell Isolator

1.1 Introduction

The ddSEQ™ Single-Cell Isolator performs rapid single-cell isolation for the Illumina® Bio-Rad Single-Cell Sequencing Solution.

Single-cell RNA sequencing (RNA-Seq) provides deep insight into cell function, disease progression, and identification of therapeutic targets in research. By profiling hundreds to tens of thousands of single cells, researchers can differentiate individual cells in heterogeneous populations. The ddSEQ Single-Cell Isolator works with Illumina Bio-Rad kits such as the SureCell™ WTA 3' Library Prep Kit. The technology utilizes disposable microfluidic cartridges to coencapsulate single cells and barcodes into subnanoliter droplets, where cell lysis and barcoding occur. RNA-Seq libraries are subsequently prepared and sequenced, and analysis is conducted via the single-cell BaseSpace™ App.

The single-cell sequencing solution:

- Processes hundreds to tens of thousands of cells per day
- Enables sensitive and unbiased characterization of transcriptional signatures
- Provides a scalable solution to accommodate a wide range of experimental designs
- Is agnostic to cell size, enabling unbiased profiling of diverse cell populations
- Delivers an end-to-end single-cell sequencing solution with simple yet powerful data analysis options

Applications and uses include:

- Cell differentiation
- Cellular reprogramming
- Subpopulation characterization
- Determining tumor heterogeneity

1.2 ddSEQ Single-Cell Isolator

The ddSEQ Single-Cell Isolator uses microfluidic cartridges and oil to coencapsulate cells and barcodes into droplets, which provide highly parallelized library prep for single-cell analysis. The system can process up to four samples in a single cartridge in under 5 minutes.

To isolate single cells, a ddSEQ Cartridge is placed in the appropriate cartridge holder. The cell mix containing samples, barcode mix, and encapsulation oil are prepared according to kit instructions and are transferred to the ddSEQ Cartridge that has been placed in the holder. The loaded cartridge is then positioned in the ddSEQ Single-Cell Isolator. There, the cell mix containing samples, barcode mix, and oil are combined within the microchannels of the cartridge to create an emulsion of monodisperse, subnanoliter-sized droplets for each of the 4 samples.

Following cell isolation, the droplets are transferred to a 96-well PCR plate for cell lysis and reverse transcription using a thermal cycler. When incubation is complete, the droplet emulsion is disrupted and sequencing library preparation follows.

The ddSEQ Single-Cell Isolator includes the components listed in Table 1. Additional required materials are listed in Table 2. Additional requirements for library preparation are listed in the individual kit instruction manuals.

Table 1. ddSEQ Single-Cell Isolator components. Catalog # refers to replacement items (quantities may be different).

Component	Description	Catalog #
ddSEQ Single-Cell Isolator	Instrument used for single-cell isolation	12004336
ddSEQ Test Cartridges	Microfluidic cartridge used to verify installation	12003862
ddSEQ Cartridge Holder	Positions and holds the ddSEQ Cartridge in the instrument for cell isolation	12004739
96-Well Cooling Block	Holds the 96-well plate on ice for transferring droplets from the ddSEQ Cartridge	12005115
Power supply	Connects instrument to power cord	Call technical support
Power cord	Connects instrument to power source	Call technical support

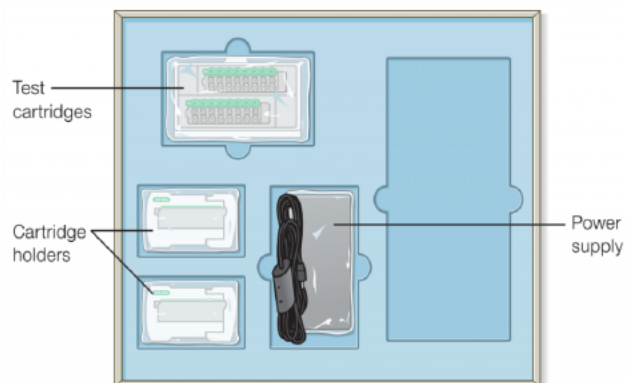


Fig. 1. ddSEQ Single-Cell Isolator and accessory box.

Table 2. Consumables and other materials required for single-cell isolation.

Component	Recommended	Catalog #
Pipets	20 μ l pipet or 8-channel, 20 μ l pipet 50 μ l pipet or 8-channel, 50 μ l pipet 8-channel, 200 μ l pipet	Rainin L-20, L8-20 Rainin L-50, L8-50 Rainin L8-200
Pipet tips	Filtered	Rainin GP-L10F, GP-L200F
96-well PCR plates	ddPCR™ 96-well plates	12001925
8-cap strips	0.2 ml Flat PCR Tube 8-Cap Strips, optical, ultraclear	TCS0803

1.3 Installation

Before handling or unpacking the ddSEQ Single-Cell Isolator, make certain that the selected site is appropriate. Place the instrument on a sturdy bench or table top, away from any instrumentation that may interfere electrically or mechanically (vibration free). Be sure the area is clean and free of excessive dust or moisture. For instrument specifications, refer to Chapter 3.

1. Open the accessory kit and verify the contents according to Figure 1.
2. Unpack the power supply and power cord.
3. Remove the Single-Cell Isolator from the box and position the instrument such that it can be easily disconnected from the power source should that become necessary for servicing the equipment. Leave 10" (25.4 cm) clear space behind and 5" (12.7 cm) clear to the right and left for proper ventilation.
4. Connect the power cord to the power supply. Connect the power supply to the back of the instrument. Power the instrument by connecting the power cord to a wall outlet.

Note: This will power up the instrument as the instrument does not have a power switch.

5. Upon powering up, confirm the door closes automatically and the two arrows of the push button illuminate green (Figure 3).

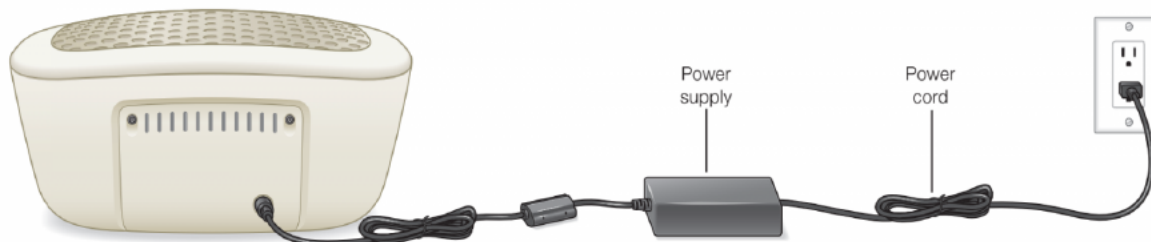


Fig. 2. Connecting the ddSEQ Single-Cell Isolator.

1.4 Instrument Test



Fig. 3. The power is on when the two arrows on the push button illuminate green.

The ddSEQ Single-Cell Isolator ships with two test cartridges to verify instrument function. These cartridges are gray and cannot be used for cell isolation. The test cartridges are reusable, but should not be used after the marked expiration date. New test cartridges can be ordered from Bio-Rad (see Table 1 for part number information). A test cartridge should be used upon initial installation of the instrument and may also be used after long idle times or when troubleshooting.

1. Remove one cartridge from its packaging by gripping the tab at the end of the cartridge.
2. Swing the latch of the cartridge holder upward until fully open.
3. Slide the cartridge into the holder. Align the green gasket on the cartridge with the green stripe on the holder.



Fig. 4. Holding and inserting a ddSEQ Test Cartridge into the cartridge holder.

- Slide the cartridge under the rails of the holder such that the cartridge is flat against the bottom, not tilted at an angle, and close the latch. Ensure the cartridge has been properly inserted by confirming the latch is in the closed position.

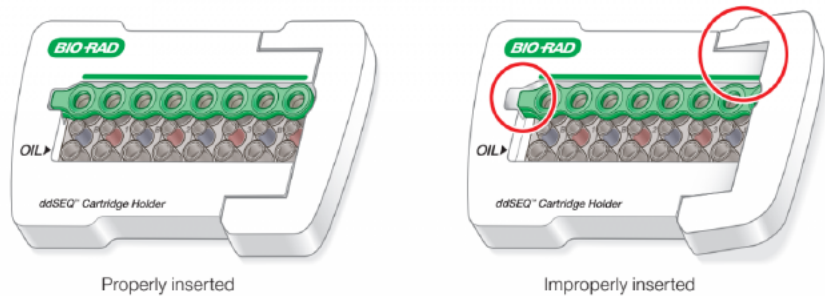





Fig.5. Proper cartridge insertion. Ensure proper insertion of cartridge by verifying latch is in the closed position (left). If latch does not close completely (right), reinsert the cartridge.

- Open the instrument door by pressing the button on the top of the door.
- Insert cartridge holder into the instrument, being careful not to open the latch when picking up the cartridge holder. Verify that the holder sits flat and does not rock from side to side. When the holder is in the correct position, both the power (left) and holder (middle) indicator lights are green (see Table 3).
- Press the same button again to close the door and initiate the test. The droplet indicator light (at right) flashes green after 10 sec to indicate the test is in progress.
- After approximately 5 min, all three indicator lights will be solid green, indicating the test run is complete and instrument is successfully installed. If the lights are not solid green, please contact Bio-Rad Technical Support for assistance.

Table 3. Status indicator lights on the ddSEQ Single-Cell Isolator. If the central LED flashes amber, the gasket on the cartridge is not providing an adequate seal. Do not rerun a cartridge if an error occurs. Contact Bio-Rad Technical Support for assistance.

			
Solid green	Power on	Cartridge holder in place	Run complete
Flashing green	—	—	Run in progress
Flashing amber	—	No seal	—
Off	Power off	No cartridge holder	Idle

2

Single-Cell Isolation

2.1 Best Practices for Loading the Cartridge

Prepare all reagents according to the instruction manual for the kit. Insert the appropriate ddSEQ™ Cartridge into the holder and follow the step-by-step instructions outlined in the kit manual.

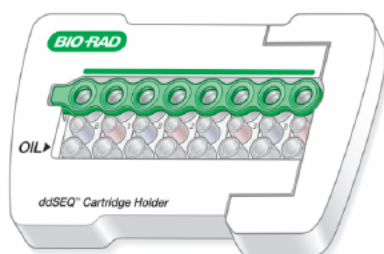


Fig. 6. ddSEQ Cartridge Holder and loaded cartridge in place.

The ddSEQ Single-Cell Isolator processes a single cartridge containing up to four samples at a time. Each run takes <5 min.



- All wells on the ddSEQ Cartridge must contain reagent prior to starting the run
- Not loading oil to the cartridge increases the risk of aerosol generation, which is a potential hazard
- Do not load sample or oil into the ddSEQ Cartridge unless it is inserted in the holder

Air bubbles can be introduced into the well and result in poor data quality. They are difficult to see, but can be avoided using the following pipetting techniques.

- Use only 20 μ l aerosol-barrier (filtered) Rainin pipet tips for reagents 20 μ l or less; do not use 200 μ l pipet tips (see Table 2)
- Gently slide the pipet tip down the side of the well at a $\sim 15^\circ$ angle until it passes over the ridge near the bottom. Holding the angle, ground the pipet tip against the bottom edge of the cell or barcode well while slowly dispensing a small portion of the sample; do not pipet directly onto the side (wall) of the well
- After dispensing about half the sample, slowly draw the tip up while dispensing the rest of the sample; do not push the pipet plunger past the first stop

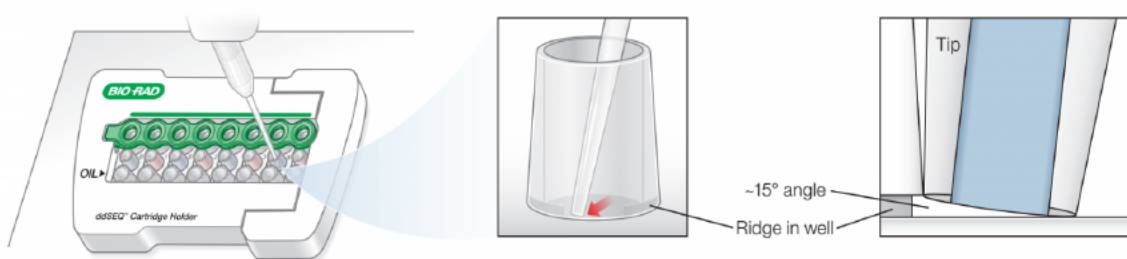


Fig. 7. Pipetting into a ddSEQ Cartridge. Hold the pipet tip at a 15° angle at the bottom of the well (middle and right panels); do not dispense sample onto the wall or side of the well.

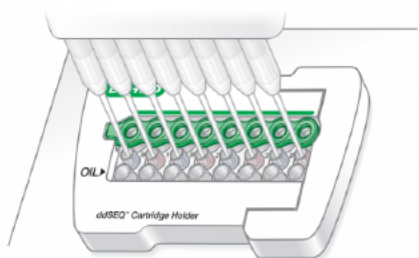


Fig. 8. Pipetting with a multichannel pipet is recommended for priming the cartridge.

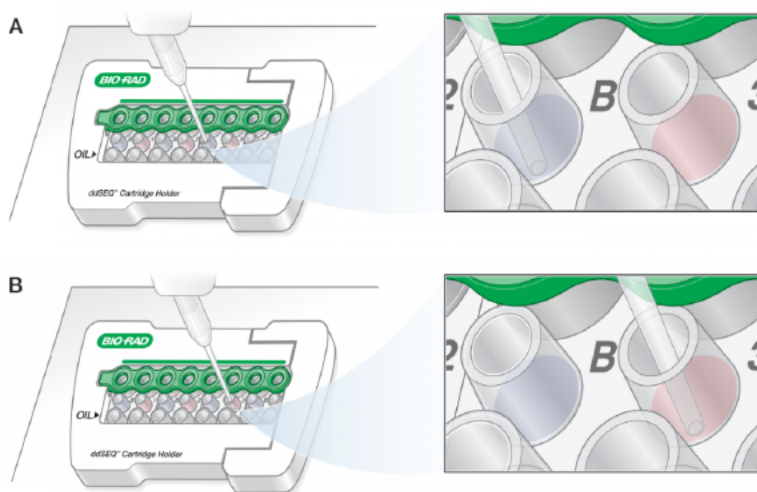


Fig. 9. Use a single channel pipet for loading the barcode mix (A) and cell mix (B) into the cartridge.

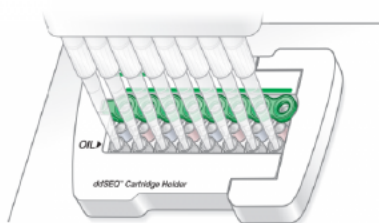


Fig. 10. Pipetting with a multichannel pipet is recommended for loading the oil into the cartridge.

2.2 Operation of the ddSEQ Single-Cell Isolator

1. Open the ddSEQ Single-Cell Isolator by pressing the button on the top of the door. Place the cartridge holder into the instrument. When the holder is in the correct position, both the power (left) and holder (middle) indicator lights are green (see Table 3).
2. Press the button again to close the door. This initiates cell isolation: a manifold positions itself over the outlet wells, drawing oil, cells, and barcode mix through the microfluidic channels, where encapsulation takes place. Droplets flow to the outlet wells, where they accumulate. The droplet indicator light (at right) flashes green after 10 sec to indicate cell isolation is in progress.
3. When cell isolation is complete (~5 min), all three indicator lights are solid green. Open the door by pressing the button. The instrument motor will continue to sound as the door opens while the run is completed. Remove the holder (with ddSEQ Cartridge still in place) from the unit. The outlet wells of the cartridge contain droplets, and the inlet wells contain a small amount of residual oil.

2.3 Best Practices for Droplet Transfer

1. Pipet the contents of the outlet wells (the droplets) into a single column of a 96-well PCR plate placed in the cooling block resting on ice.
2. Seal the PCR plate with strip-tube caps immediately after transferring droplets to avoid evaporation.
3. Once droplets are removed from the empty ddSEQ Cartridge, remove the cartridge from the holder and discard.

For best results, use the following pipetting techniques to transfer droplets from the ddSEQ Cartridge into a 96-well plate:

- Place a new 96-well plate into the 96-well plate cooling block and set on packed ice
- Use an 8-channel manual L-50 pipet with 200 μ l filter tips, as the L-50 pipet allows for slower pipetting and finer control of droplet pickup. Do not use an L-200 pipet
- Place the cartridge holder on a flat surface and slide the pipet tips straight down into each of the 8 top wells until they reach the junction where the side wall meets the bottom of the well, and then tip them to a $\sim 30\text{--}45^\circ$ angle. Do not position the pipet tip in a vertical orientation or against any flat surface of the well; do not allow the tips to be flat against the bottoms of the wells
- Pipet slowly and apply a stable resistive force to the plunger to draw and aspirate droplets smoothly into and out of pipet tips
- Slowly draw 43 μ l of droplets into the pipet tip (should take ~ 10 sec, and ~ 5 μ l air is expected)
- To dispense droplets into the 96-well plate, position the pipet tip along the side of the well — near, but not at, the bottom of the well — and slowly dispense the droplets (~ 10 sec)

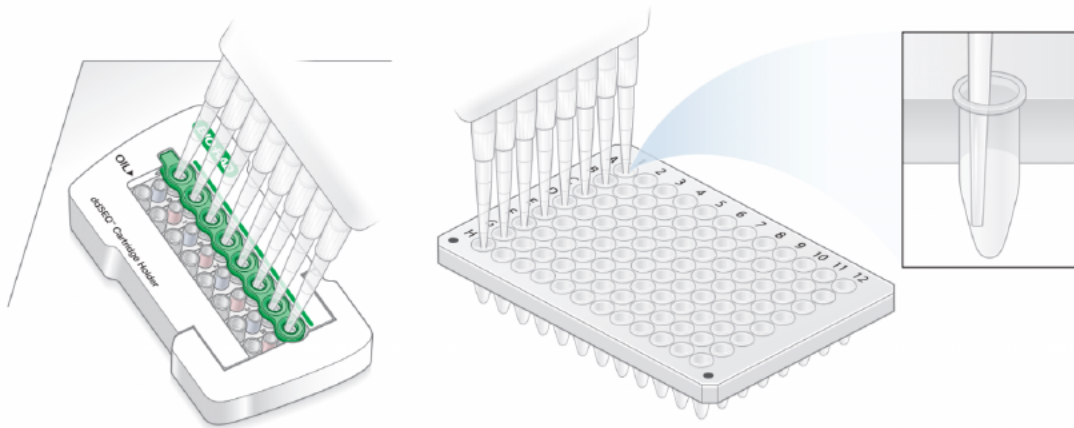


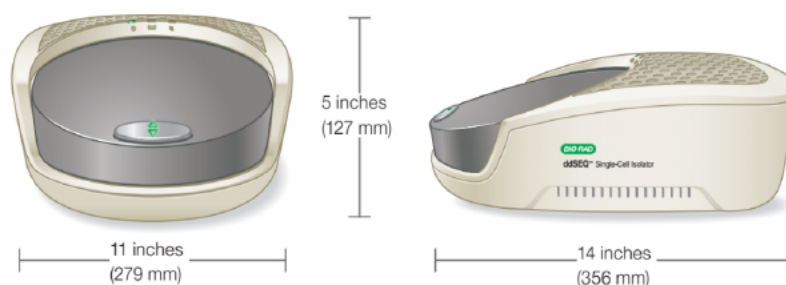
Fig. 11. Pipet droplets slowly from the ddSEQ Cartridge and transfer to a 96-well plate in the cooling block on ice, dispensing the droplets slowly along the side of the well.

To prevent evaporation and contamination with particulates, seal the plate with 8-cap strips immediately. Keep the plate on ice until transferred to the thermal cycler (within 30 min).

3

Specifications and Maintenance

3.1 Specifications



Weight	10 lb (4.5 kg)
Size (W x D x H)	11 x 14 x 5 in (28 x 36 x 13 cm)
Electrical requirements	100–240 V, 50/60 Hz, 60 W; voltage fluctuations not to exceed +10% of ratings (for external power supply provided)
Temperature	18–30°C
Altitude	0–6,560 ft (0–2,000 m)
Humidity	85% max (noncondensing)
Pollution degree	2 (indoor use)
Installation category	II (external power supply plugs into standard AC receptacle)
Ventilation requirement	5 in (12 cm) left and right of machine and 10 in (25 cm) behind should be unobstructed for proper ventilation

3.2 Maintenance

Surfaces of the ddSEQ™ Single-Cell Isolator may require general cleaning. Use deionized/distilled water for general wipe-down with a slightly dampened cloth. For decontamination, use 10% bleach followed by 70% ethanol and/or deionized/distilled water. Do not use acetone or tap water.

Inspect equipment regularly for damaged external components or wiring. Do not use if damaged.

Apply standard SDS or MSDS (material safety data sheet) and Occupational Safety and Health Administration (OSHA) practices when handling and disposing of generated waste.

Illumina Bio-Rad Single-Cell Sequencing reagents are based on fluorinated hydrocarbon chemistry and should be disposed of in accordance with institutional, state, and local regulations. These nonflammable fluids are inert and have low environmental impact and low toxicity.

Droplets made with Illumina Bio-Rad reagents have antimicrobial properties, but microbial growth is possible. The waste profile should contain the following: fluorinated hydrocarbons, water, protein, and nucleic acids.

Do not replace detachable power cord with an uncertified or an inadequately rated cord.

Ordering Information

ddSEQ™ Single-Cell Isolator

Catalog #	Description
12004336	ddSEQ Single-Cell Isolator , includes instrument and associated component consumables for single-cell NGS applications



**Bio-Rad
Laboratories, Inc.**

*Life Science
Group*

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