MD-Box-Lab User Manual

COYOTE BIOTECH

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Index

Introduction

“MD-box-lab”, the only one nucleic acid detection carrying case that can be claimed as a regular bag on the flights. More importantly all the devices inside the box are 1-2 kg and portable, which are compatible with a battery pack or car charger. The integrated solution of “Lab in a box” is an ideal choice for the diagnosis of Ebola and Dengue fever, etc.

Features & Benefits

* The only one nucleic acid detection carrying case
* Compatibility: flexible Open System
* High sensitivity& specificity
* User friendly
* Field test

All the device in the box are compatible with 12V DC power or batteries.Using MD-box-lab can greatly improve the efficiency of the experiment, save space costs; reduce the difficulty of the experiment. It can also make on line detection operation of nucleic acid more simple and quick.

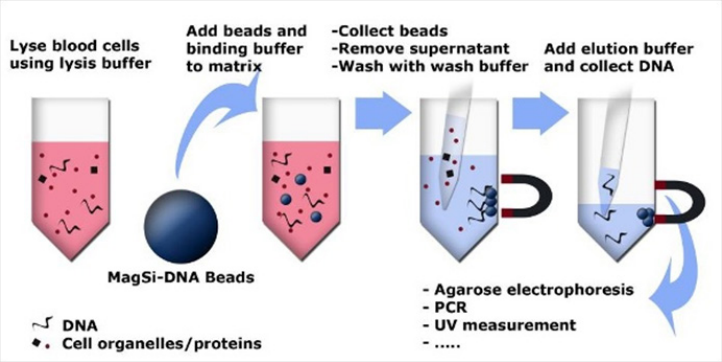
The MD-lab-box can be used with different types of nucleic acid extraction kit based on the magnetic beads method, to meet the nucleic acid extraction and purification purposes of different samples. This system is open for most of commercial real time PCR kit for quantitative detection purpose.

Apart from the full solutions for the molecular diagnostic purpose including devices, consumables and protective suit, the MD-Box-lab could also be customized order to fill your different need.

Part I Sample preparation (magnetic beads)

Magnetic beads are a simple and reliable method of purifying genomic, plasmid and mitochondrial DNA. Under optimized conditions, DNA selectively binds to the surface of magnetic beads, while other contaminants stay in solution. Purified DNA can then be used directly in molecular biology applications such as sequencing or restriction digests. The major advantage of this method is that there is no need for centrifugation or vacuum manifolds, which can be a bottleneck in many automated processes.

Magnetic bead extraction workflow:



Part II Nucleic acid detection: Mini8 Real-Time PCR system

Real-time PCR, also called quantitative PCR or qPCR, can provide a simple and elegant method for determining the amount of a target sequence or gene that is present in a sample.

A real-time polymerase chain reaction is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR), which is used to amplify and simultaneously detect or quantify a targeted DNA molecule. The key feature is that the amplified DNA is detected as the reaction progresses in "real time". This is a new approach compared to standard PCR, where the product of the reaction is detected at its end.

Two common methods for the detection of products in quantitative PCR are:

(1) non-specific fluorescent dyes that intercalate with any double-stranded DNA, and (2) sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence to quantify messenger RNA (mRNA) and non-coding RNA in cells or tissues.

There are numerous applications for quantitative polymerase chain reaction in the laboratory. It is commonly used for both diagnostic and basic research. Uses of the technique in industry include the quantification of microbial load in foods or on vegetable matter, the detection of GMOs (Genetically modified organisms) and the quantification and genotyping of human viral pathogens.

Mini8 Real-Time PCR system is a robust, unique and as precise as any large-scale apparatus. Our system provides you with precise test results quickly and cost effectively anywhere, anytime.
Mini8 Real-Time System offers the smallest footprint of any system available.

Notes

Two common detection methods:

(1) SYBR Green ® /EvaGreen @ /LC Green dyemethod
(2) Taqman @probe method

Nucleic acid template types:
(1) the template is DNA: qPCR
(2) the template is RNA: qRT-PCR

Therefore, the following four combinations of test kits are applied:
A.qPCR SYBR Greendye
B.qPCRTaqman probe
C.qRT-PCR SYBR Greendye
D.qRT-PCR Taqman probe

Application fields

* Clinical diagnosis: hepatitis, AIDS, avian influenza etc
* Animal disease detection: avian influenza and Newcastle disease, foot-and-mouth disease etc
* Food safety: food-borne microorganisms, food allergens, transgenic, enterobactersakazakii detection etc.
* Scientific research: quantitative study of medicine, agriculture and animal husbandry, biological molecular biology.

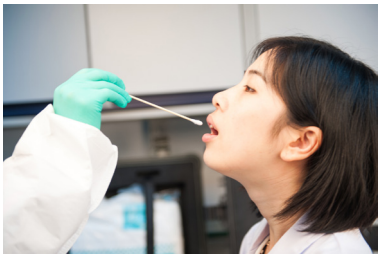
Workflow

Using two MD-Box-Labs to do the full molecular diagnostic

STEP 1: Wear protective suit

STEP 2: Alcohol prep pad

STEP 3: Sample collection



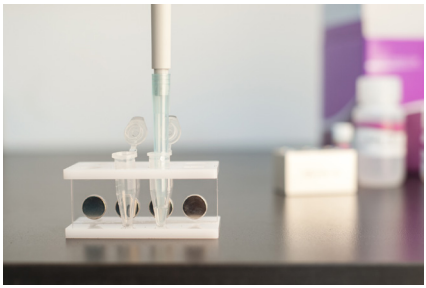
Throat swab

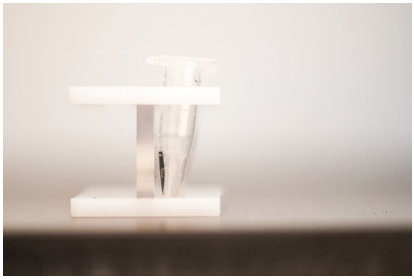
Blood sample

Tissue sample

STEP 4: Nucleic acid extraction

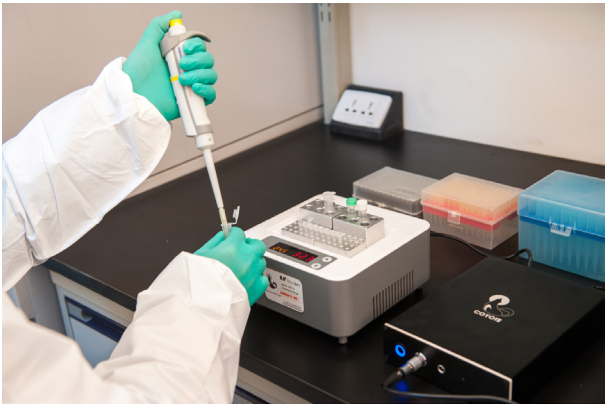
Extract the RNA/DNA sample by using the extraction kit.
Note: read the appendix the extraction kit instruction





STEP 5: PCR mix preparation

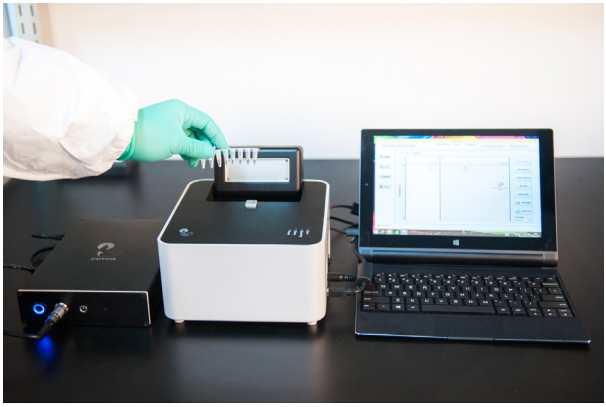
Prepare the PCR mix according to your detection kit manual



STEP 6: Running PCR assay

Turn on the Mini8 Real-Time PCR System. Set up the thermal profile and plate date

Note : Mini8 Real-Time PCR System driver and software are pre installed in the PC tablet. Read the appendix of the Mini8 Real-Time PCR System for the instruction.



STEP 6: Results analysis

Appendix -User manual for the MD-Box-Lab devices and extraction kit

Tissue Grinder Model G10/G20

G10 Tissue Grinder is a motor-driven grinder for re-suspending pellets or disrupting soft tissue in microcentrifuge tubes. The motor is powered by a 3.7V battery and can be used cordlessly for up to 10 hrs.

Operation

Take the grinder out. Place the pestle on the pestle adapter. Put the pestle into the microcentrifuge tube, and use the bottom button to start mixing. Release the button after the mixing operation is completed.

Charging the battery

Screw off the bottom button from the grinder. Take out the battery. Put the battery on the charger. Battery charging completes after the indicator light turns green.

Specifications

Power Supply: 3.7 lithium battery
Speed: G10: 12000 rpm G20: 1200 rpm
Continuously working for 10 hrs
Dimensions: 155mm*Φ25 mm
Net Weight: 0.2 kg

Pipette

The pipette is for general laboratory liquid dispensing. All pipettes have been certified using gravimetric with distrilled water at 22°C. Coyote offers four singles channel pipettes with volumes form 0.1µl to 1000µl.

Operation

Note: Pipettes should be checked prior to use to ensure the tip of the cone is clear of debris.

Volume Selection

The volume of the pipette is shown in the display window located on the handle. The volume can be set by turning the Thumb Button clockwise or counter clockwise.

Sealing/Ejecting Pipette Tips

Press the appropriate pipette tip firmly onto the shaft of the pipette. When the seal is tight, a visible ring forms between the tip and the shaft. Each pipette has a tip ejector. The tip ejector must be firmly pressed downwards to ensure contact of the ejector with the top of the pipette tip.

Specifications

Range: 0.1-2.5µl; 2-20 µl; 20-200 µl, 100-1000µl

Dry bath

H_2O^3-100C is a thermoelectric (Peltier) heating/chilling device. This unit is designed for handling and storing small sample tubes at a specific temperature ranging from 0~100ºC. The samples on its aluminum sample blocks can be automatically maintained at set point when connected to the electricity outlet via the provided 12 V DC power adaptor. It can work either with cord or cordlessly. This device is used for loading and storing samples, enzymatic reactions, and molecular cloning experiments. H_2O^3-100C has a modular design of blocks.

Operation

Temperature Display: While the device is on, the LED display shows actual temperature on the right side and shows set point on the left side.

Temperature setting: press the ▲ and ▼ key to set the temperature.

Specifications

Model: H2O3-100CB
Temperature range: 0-100℃
Operation ambient temperature: 8-30℃
Display precision: 0.1℃
Power Supply: 12V DC 7A
Temperature precision: ±0.5℃
Temperature evenness: ±0.5℃
Dimension: 185×185×90 mm
Working area dimensions: 108×72mm
Net Weight: 1.85kg
* Average Heating Rate: 11℃ /min(25-75℃)
* Average Cooling Rate: 2.5℃ /min(25-4℃)
* Room Temperature at 25℃ with 1*0.2ml blocks

Magnetic separator

The magnetic separator is designed for 1.5, 2ml snap cap microcentrifuge tubes. The rack securely holds up to 8 tubes at a time. The magnetic beads are pulled up to the sides of the tubes so that the liquid can be evacuated by simply flipping the rack upside down and pounding the rack on blotting paper to remove all liquid drops.

Each tube position is adjacent to both a high and low magnet in order to provide good bead separation in a wide range of volumes of solution to be separated. No matter the volume in the wash or reaction liquid, the beads are quickly pulled to the side of the tube.

Specifications

Model: C1008
Capacity: 8 samples
Adaptive microcentrifuge tube: 1.5,2.0 ml

Mini 8 Real-time PCR system

Mini8 Real-Time PCR system is designed for molecular diagnostic market. It has a small footprint, with high detection sensitivity. The machine has two channels, one is SYBR/FAM, and the other is ROX. The sample capacity is 8 × 0.2mL PCR tubes, and it is compatible with any commercial SYBR or TaqManqPCR kits. The user interface on the computer is friendly and very simple to operate.

This fluorescence dye preference can be specially ordered per customer's request. This product equips 12 V DC power supply, that is compatible with a car charger and a battery pack.

Operation

- Connect Mini8
- Install the Mini8 Driver
- Turn on the Mini8 System
- Mini8 System Workflow

1 Prepare the sample plate, load it into the Mini8, and close the lid.

2 Double-click the Mini8 icon on desktop to open thesoftware.

3 Define and name the experiment by selecting the application, detection chemistry, starting material and specific method for your application.

Tip: to use a pre-defined thermal profile and plate layout for your experiment,click and select one of the template experiments saved on your notebook.

4 Review the thermal profile and adapt it if needed.

5 Set up the plate layout by defining assays, samples, andstandards and assigning them to wells.

6 Start the run. The Monitor Run tab opens.(Do not open the lid while a run is in progress.This will corrupt the data.)

7 When the run is complete, open the Mini8 lid. Remove the plate from the block.Dispose of any hazardousmaterials in biohazard,caustic material, or other appropriate containers,according to your local safety regulations.

Note: The mini8 driver and software is pre-installed in the PC tablet. For the detail information, please read the Mini8 Real-Time PCR System manual for your reference.

Specifications

Illuminant: LED
Detection: photodiode
Heating/chilling model: semi-conductor
Max temperature change rate: 3℃ /s
Temperature evenness: ±0.2℃
Temperature precision: ±0.2℃
Temperature range: 4-100℃
Capacity: 8 wells
Reaction Volume: 15-150μL
Preheating time: 1min
Detection sensitivity: 1 copy
Detection Channel: 470/520nm(SYBR/FAM） and 565/625nm(ROX/ Texas Red)
Dimension: 205×190×98 mm (L×W×H)
Net Weight: 2.1kg
Power Supply: 12V,10A
PC requirement: WIN2000;XP; WIN7;WIN8

Ambient Temperature:
operation temp:15-30℃
storage temperature:10-60℃;

Ambient humidity:
operation humidity: 15-90% relative humidity:
Storage humidity: 5-95% relative humidity

Battery Pack

BATT-3 is an universal battery pack, and can be used with our Mini8 Real-Time PCR System, Dry Baths, Slim PCR Cyclers and G50motor-driven tissue grinders. It is an ideal accessary for the field applications. Also, it can be used as an alternative power source for a lab when the electricity is not stable. The power input goes from the power adapter through the battery pack to the equipment. When the power is shut off for some time, the battery pack can keep the equipment working for several hours.

Features

* 12V DC, portable
* Ideal choice for field application
* Automatically stop charging while the battary pack is fully charged
* Charging indicator light off shows the fully chaged status
* Personalized design of the battary capacity indicator

Operation

1) Read the user manual carefully before your use.

2) Take the Battery Pack out, place it on a flat surface.

3) Charging: Plug a 12V DC power adaptor into the “Power Input” port

* Automatically stop charging while the battary pack is fully charged
* Charging indicator light off shows the fully chaged status

4) Power Indicator: Press the power indicator button next to the 4 indicating lights to check the remaining capacity of the Battery Pack. NO. 1-4 stand for 25%, 50%, 75%, 100% percentage of remaining capacity. When the power indicator off, please charge the battery before using. (Figure 1)

5) Supplying the power to Coyote’s devices: Turn on the switch, link the Battery Pack.

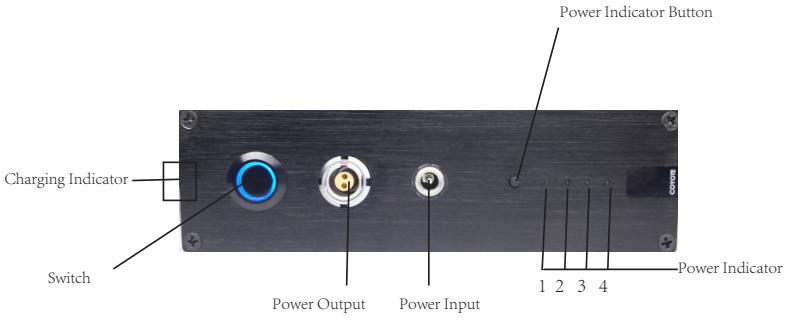


Figure 1: batt3 battery pack

Caution

* Power output line (Grey) is for the PCR series products (Figure 3)
* Power output line (black) is for the other products (e.g. dry bath) (Figure 3)
* Please insert the power output line red dot to match the red dot on the device (Figure 2)
* Hold the arrow point to remove the power output line (Figure 2)
* Battery pack are not allowed to charge and discharge together
* For long term store, please place the battery pack in a dry and cool place and remain at least 50% capacity
* Prevent water intake
* Avoid working under high temperature/high humidity

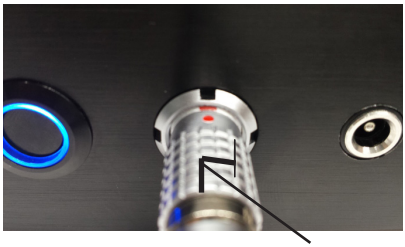


Figure 2

Figure 3

Specifications

Model: Batt-3
Battery:lithium
Capacity: 130Wh
Power input: 12V, 10A DC
Charging time: 5hr
Power output: 12V DC, max 10A
Working hour: read power supply reference
Dimension: 152*44*200mm
Weight: 1750g
Operation ambient humidity: 0-35℃

Mini Centrifuge

Operation

1 Open the lid and switch on the on/off switch is located back of the unit.
2 Symmetrically load centrifuge tubes to rotor.
3 Close the centrifuge lid to begin running.
4 Switch off, the rotor will gradually stop.
5 Open the lid and remove samples until the rotor extremely stopped.

Specifications

Model: C1008
Max Speed: 7000 rpm
Max RCF: 2680 xg
Rotor: 8 × 0.2/0.5/1.5/2.0ml Tubes
Centrifuge tube: 16 x 0.2ml PCR tube; 2 × 0.2ml 8 PCR tubes; 2 standard slides
Runtime: Continuous
Motor: DC motor
Power Supply: 12V DC
Noise Level: ≤ 45 dB
Dimension: (D×W×H):150 x 150 x 117 mm
Net Weight: 0.5 kg

PC tablet

Processor: Intel Atom Z3745 Processor (1.33GHz 1066GHz 2MB)
Operating system: Android 4.4
Display: 10.1" IPS LED LCD Touch (1920x1200)
Memory: 2.0GB LPDDR3 1066 MHz
Hard Drive: 16GB EMMC
Network Card: 802.11abgn WLAN
Bluetooth: Bluetooth Version 4.0
Warranty: One year
Battery: 3 Cell 9600mAH Li-Cylindrical
Camera: Front 1.6MP HD, Rear 8MP

Magnetic Beads Virus DNA/RNA Kit Manual Protocol

Introduction

The Magnetic Beads Virus DNA/RNA Extraction Kit was designed specifically for efficient purification of viral DNA and viralRNA from cell-free samples such as serum, plasma, body fluids and the supernatant of viral infected cell cultures. ViralDNA/RNA is bound to the surface of the magnetic beads and released using a proprietary buffer system. The Magnetic Beads Viral DNA/RNA Kit can be easily adapted to automated magnetic bead separation instruments and workstations. The purified DNA/RNA can be used in qPCR and qRT-PCR assays.

Advantages

• Easily adapted to automated magnetic bead separation instruments and workstations
• Sample: up to 200 µl of virus samples (plasma, serum, body fluid or the supernatant of viral infected cell cultures)
• Operation time: within 1h (manual)
• Storage: dry at room temperature (15-25ºC) for up to 12 months

Caution

During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.
Disposable/non-disposable glassware, plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.

Components and Storage

Component	DP438	Shipping	Storage

Buffer RLCK	15ml	room temperature	dry at room temperature (15-25ºC) for up to 12 months
Buffer PW1	18ml	room temperature	dry at room temperature (15-25ºC) for up to 12 months
Buffer PWII	12ml	room temperature	dry at room temperature (15-25ºC) for up to 12 months
Carrier RNA	310ug	room temperature	dry at room temperature (15-25ºC) for up to 12 months
ProteinaseK	1ml	room temperature	dry at room temperature (15-25ºC) for up to 12 months
MagAttract Suspension G	1ml	room temperature	dry at room temperature (15-25ºC) for up to 12 months
RNase-Free ddH2O	1ml	room temperature	dry at room temperature (15-25ºC) for up to 12 months
RNase-Free ddH2O	8ml		dry at room temperature (15-25ºC) for up to 12 months

Carrier RNA buffer preparation

Add 310ul of RNase-free Water to 310 ugCarrier RNA then vortex or use pipette to mix. Ensure Carrier RNA is completely dissolved to obtain a working solution of 1 µg/µl. Once it is dissolved completely, centrifuge for a few seconds to spin the mixture down. Divide the Carrier RNA solution into convenient volumes in several RNase-free 1.5 ml microcentrifuge tubes and store at -20ºC.

Do not freeze and thaw Carrier RNA solution more than 3 times.

Carrier RNA working solution preparation

Prepare the carrier RNA working solution according to your sample quantity (see the table below as your reference). Mix the RLCK buffer with Carrier RNA buffer solution to get the Carrier RNA working solution. Do not vortex the Carrier RNA working solution.

Quantity	RLCK (ml)	Carrier RNA	25Quantity	RLCK(ml)	Carrier RNA
1	0.31	3	13	4.03	39
2	0.62	6	14	4.34	42
3	0.93	9	15	4.65	45
4	1.24	12	16	4.96	48
5	1.55	15	17	5.27	51
6	1.86	18	18	5.58	54
7	2.17	21	19	5.89	57
8	2.48	24	20	6.2	60
9	2.79	27	21	6.51	63
10	3.1	30	22	6.82	66
11	3.41	33	23	7.13	69
12	3.72	36	24	7.44	72

Magnetic Beads Virus DNA/RNA Kit Protocol Procedure

1. Add 200ul serum/plasma/body fluids to the 1.5ml microcentrifuge tube.
2. Add 15ul MagAttract Suspension G to the centrifuge tube

Note: vortex magbeads to ensure they are in suspension prior to initial use.

3. Add 20ul Proteinase K to the microcentrifuge tube
4. Add 300ul Carrier RNA working solution (see table above for the preparation method) to the sample. Close the lid and shake 10

sec for mixing.

Note: when the sample quantities is large, please premix the 300ul Carrier RNA working solution with 20 Proteinase K for each sample. It is advised to prepare the Carrier RNA working solution before use.

5. Incubate the tube at room temperature for 10min. Gently shake and mix the solution every 3min for 10sec to ensure the magnetic beads combine to the nucleic acid.

6. Place the tube in the magnetic separator for 1min or or until MV Magnetic Beads have pelleted then remove and discard the cleared supernatant.

7. Remove the tube from the magnetic separator and add 500ul PW1 (ethanol was added), shake the tube for 1min.

8. Place the tube in the magnetic separator for 1min or or until MV Magnetic Beads have pelleted then remove and discard the cleared supernatant.

9. Remove the tube from the magnetic separator and add 500ul PW2 (ethanol was added), shake the tube for 1min.

10. Place the tube in the magnetic separator for 1min or or until MV Magnetic Beads have pelleted then remove and discard the cleared supernatant.

11. Repeat step 9 and 10 once.

12. Place the tube in the magnetic separator, open the lid to dry at 56 °C for 5-10min.

13. Remove the tube from magnetic separator, add 100 ulRNnase-Free ddH2O, Vortex/ Shake at for 56 °C

14. Place the tube in the magnetic separator for 2 min or or until MV Magnetic Beads have pelleted then transfer the nucleic acid solution into a new tube.

Packing List

MD-Box-Lab (Device)

Item	Model	Quantity	Accessory	Quantity
Battery Pack	BATT-3	1	Main Body 12V 5A DC power adaptor Power Line Power output line (grey) Power output line (black)	1 1 1 1 1
Eppendorf Pipettor	0.1-2.5 µl 2-20 µl 20-200 µl 100-1000 µl	1 1 1 1		
Dry Bath	H_2O^3-100C	1	Block cover 0.2ml block 1.5ml block 2.0ml block Adapter 12V 7A Power line	1 2 2 1 1 1
Real-Time PCR System	Mini8	1	adaptor12V 10A power line Data line DVD	1 1 1 1
Tissue Grinder	G10	1	Charger Rechargeable lithium battery 3.7V/2.2Ah Plastic pestel Stainless steel pestel Rubber gasket	1 2 50 1 2

Magnetic Separator	8×1.5/2.0ML	1	NA	NA
Centrifuge	C1008	1	1.5ml Rotor 0.2ml Rotor Power adaptor 12V 3A Power line	1 1 1 1
PC tablet	Lenovo YOGA Tablet 2-1051F	1	Keyboard Power adaptor USB data line	1 1 1
User Manual	MD-box-lab MD-box-lab work flow	1 1	NA	NA

MD-Box-Lab (Consumable and protective suit)

Item	Model	Quantity	Usage
IceBox	Biocision Ice box	1	Reagent storage
Protective suit	Microguard protective clothing Microguard gloves Shoe cover 3M Mask UVEX 9405-714 blinder	2 4 4 4 2	Biological safety protection
Tips	Axgen 10ul Axgen 200ul Axgen 1000ul	1box 1box 1box	Adding sample
Tubes	Axgen PCR stips of 8 well Axgen caps of 8 well Axgen microcentriguge tube	1bag 1bag 1bag	Adding sample
Throat swab		1box	Sample collection
Extraction kit	Magnetic Beads Virus DNA/RNA Kit	1	Nucleic acid extraction

Acknowledgement

At the point of finishing this manual, I'd like to express my sincere thanks to all those who have lent the hands in this MD-Box-Lab development.

Firstly, would like to thank our CEO Sabrina Li who brings up this idea to develop this product.

Secondly, would like to thank all the colleagues who have make effort in this project: Chaoming Tang; Kun yang; Lanlan Jiang; HuiyingFeng; Xinzhao Shen; Changhui Shan, etc…

Our Service

Our sales support staff can provide information on pricing and give your quotations. we can take your order and provide delivery information on major equipment items or make arrangements to have your local sales representative contact you.

Our service support staff can supply technical information a about proper setup, operation or troubleshooting of your equipment. we can fill your needs for spare or replacement parts or provide you with on-site service.

Coyote Bioscience standard product warranty

This MD-Box-Lab carries a one-year warranty. The warranty period starts two weeks from the date your equipment is shipped form our facility. This allows for shipping time so the warranty period go into the effect at approximately the same time your equipment is delivered. The warrantly protection extends to any subsequent owner during the one year warranty period.

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