For sales rep training

Mycoplasma Gene Detection Kit Myco Finder (Product Code: 69202)

Operation Manual

Kit Composition (Detection Reagent)

Mycoplasma Gene Detection Kit, Myco Finder
(48 tests)

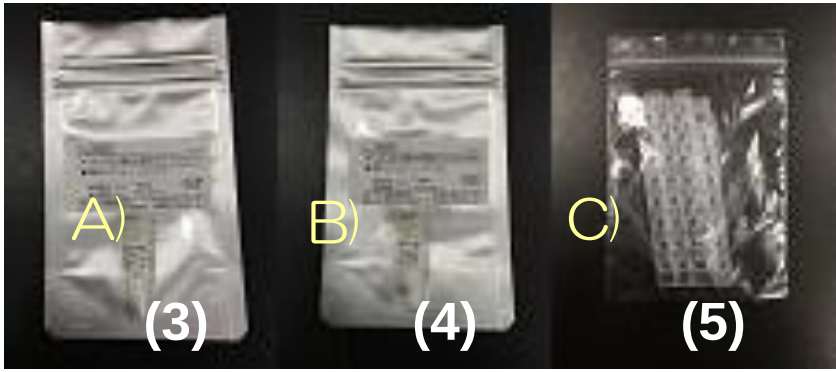
[Appearance]

(1)

(2)

[Constituent reagents]

(1) Test strip A (white) 6
(2) Test strip B (transparent) 6
(3) Positive control (2 x 10^3copies/μL) 1
(4) Negative control (DNase Free Water) 1
(5) Flat cap 6



Tools and equipment necessary for measurement

- Micro pipet (Several types: so that 5~1000 µL can be measured to be taken)
- Filter tip (Sterilized, DNase･RNase Free)
- Mixier
- Heat block (Water Bath can also be used)
- Centrifuge (Capable of maximum speed 20,000 x g)
- 1.5 mL Micro tube
- Real-time PCR system (Capable of detecting FAM + HEX or ROX)

Micro pipet

Heat block

Real-time PCR

Centrifuge

14,000 rpm is required

1.5 mL Micro tube

Mixer

Reagents for DNA Extraction

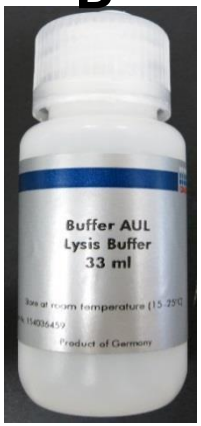
DNA extraction is performed before using the mycoplasma gene detection kit.

- **Introduction of QIAGEN QIAamp UCP DNA Micro Kit**
  Reagents to be used:

A : Proteinase K
B : Buffer AUL
C : QIAamp UCP MinElute Column
D : Buffer AUW1
E : Buffer AUW2
F : Buffer AUE
G : Collection Tube

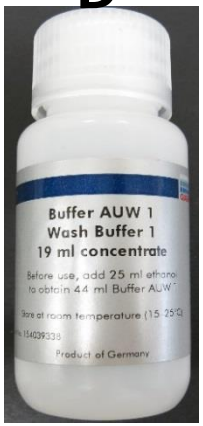
A

B



C

D



E

F

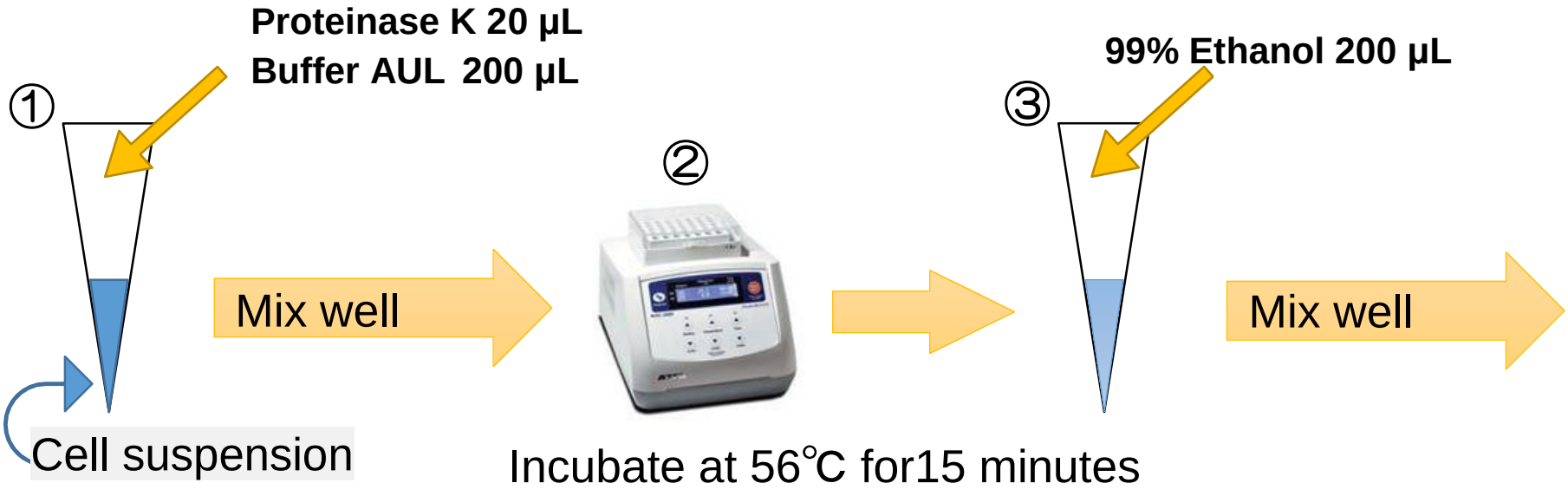
G

Note) Certain reagents need to be prepared before use. Please follow the instructions on the respective bottle.

DNA Extraction Procedure (1)

[Extraction using QIAamp UCP DNA Micro Kit (1)]

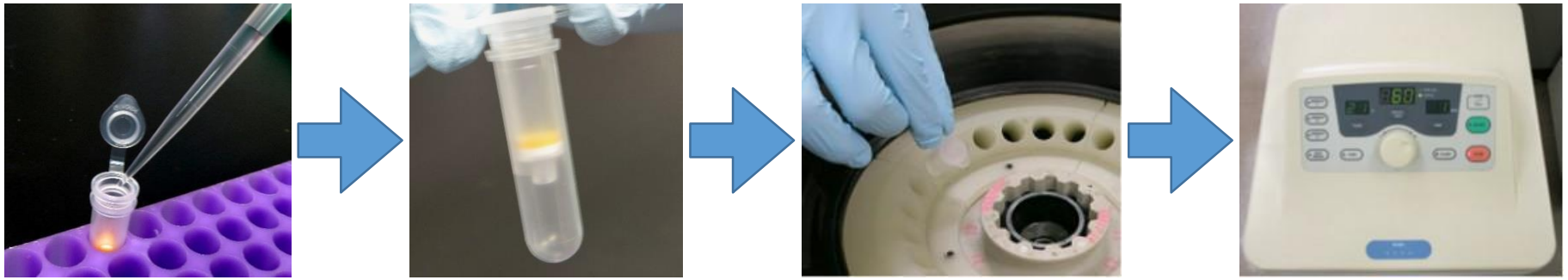
1) Add **20µL** of **Proteinase K and 200µL** of **Buffer AL** to a 200µL of cell culture or a **200µL** of **cell suspension** prepared by concentrating cell culture by centrifugation, and mix well by a mixer.
   Note) Mix well until the solution is homogenized.
2) Incubate at 56℃ for 15 minutes. After incubation, spin down to get rid of the liquid on the lid
3) Add **200µL** of **99 %** ethanol and mix well by a mixer.
   Note) Mix well until the solution is homogenized.



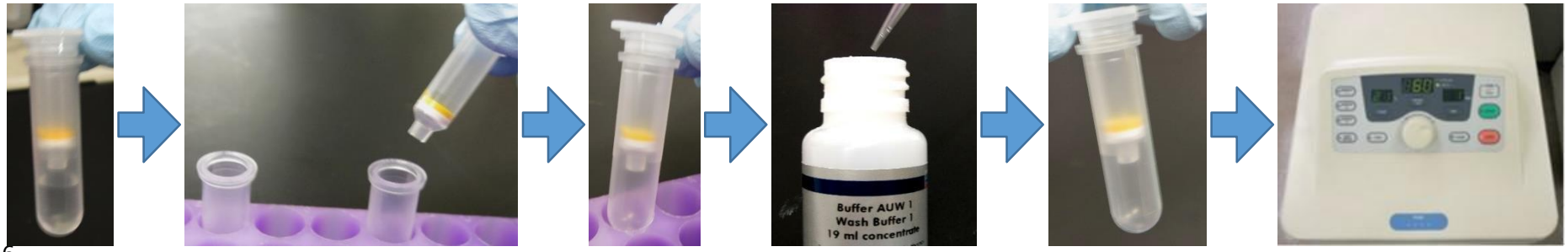
DNA Extraction Procedure (2)

[Extraction using QIAamp UCP DNA Micro Kit (2)]

4) Move all into **QIAamp UCP MinElute Column** and centrifuge at 6,000 × g (8,000 rpm) for 1 minute.



5) Place the **QIAamp UCP MinElute Column** into a new collection tube and add **500µL** of **Buffer AW1**. Centrifuge at 6,000 × g (8,000 rpm) for 1 minute.

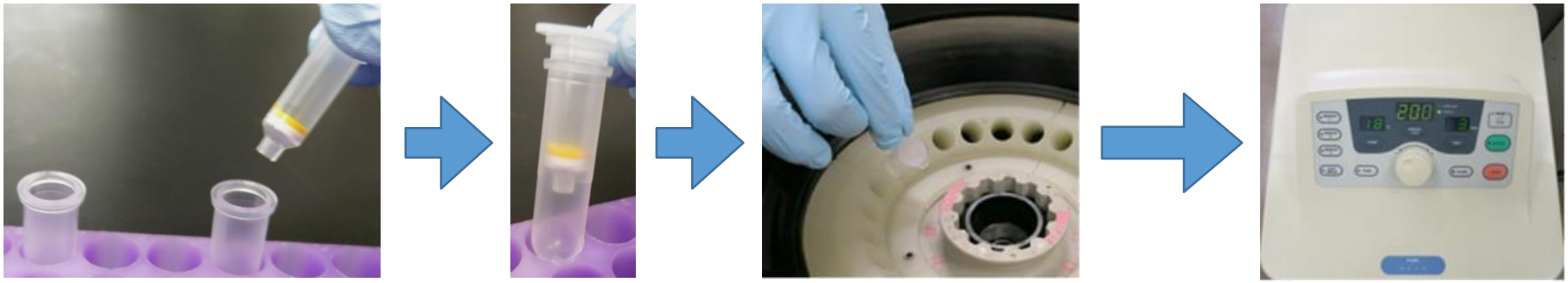


DNA Extraction Procedure (3)

[Extraction using QIAamp UCP DNA Micro Kit (3)]

6) Place the **QIAamp UCP MinElute Column** into a new collection tube and add **500µL** of **Buffer AW2.** Centrifuge at 20,000 × g (14,000 rpm) for 3 minutes.

7) Place the **QIAamp UCP MinElute Column** into a new collection tube and centrifuge the empty **QIAamp UCP MinElute Column** at 20,000 × g (14,000 rpm) for 1 minute.

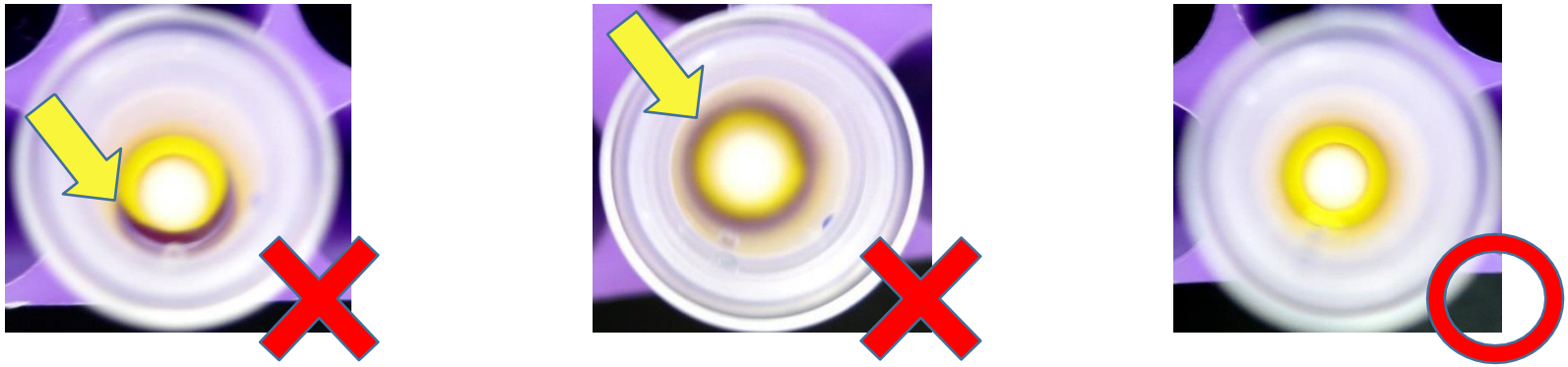


DNA Extraction Procedure (4)

[Extraction using QIAamp UCP DNA Micro Kit (4)]

8) Confirm that there is no remaining liquid in the **QIAamp UCP MinElute Column**

Note) The liquid in the example below is colored in blue for better visibility but actual liquid is transparent.



9) Place the **QIAamp UCP MinElute Column** into 1.5mL tube and add **100µL** of **Buffer AUE**. Incubate at room temperature (15 to 25℃) for 5 minutes. Centrifuge at 6,000 × g (8,000 rpm) for 1 minute and elute the sample.

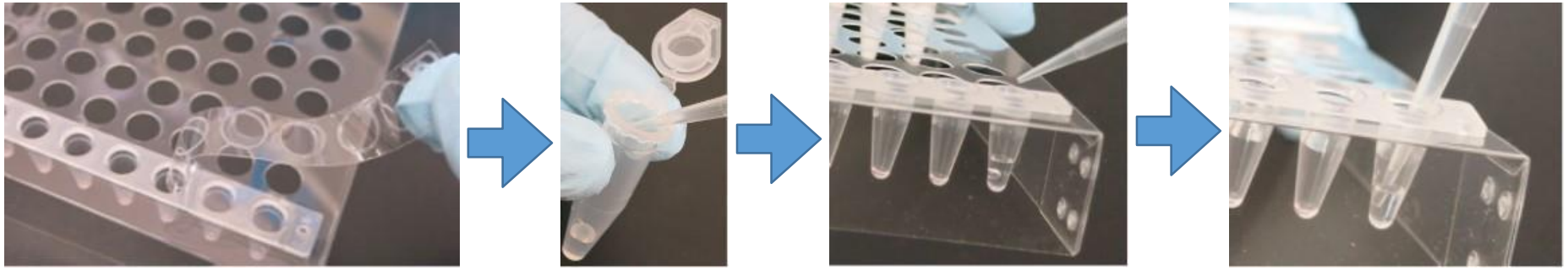
Preparation of controls

○Prearation of positive control
⇒Add 1μL of positive control to 24μL of negative control.

○Preparation of negative control
⇒Use the 25 μL of negative control as is.

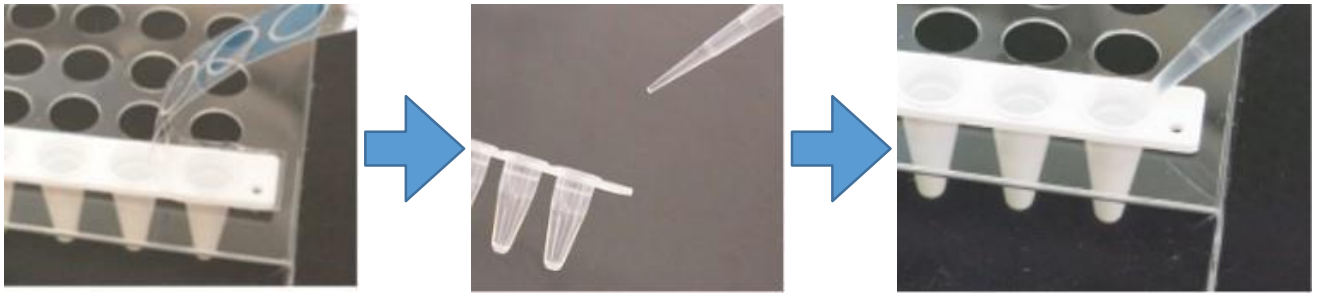
1) Take the necessary quantity of test strip A and test strip B out of the aluminum bag.

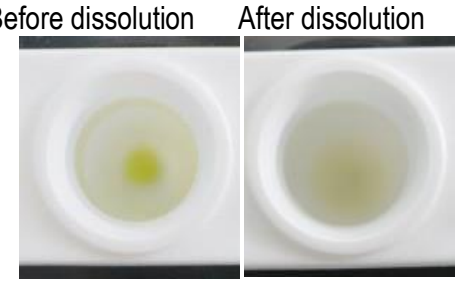
2) Peel off the film from test strip B and add 25 μL each of the sample from DNA extraction, positive control, and negative control into respective wells, and pipet gently for 20 times to dissolve the solidified reagent. Dilute the sample as necessary before use.



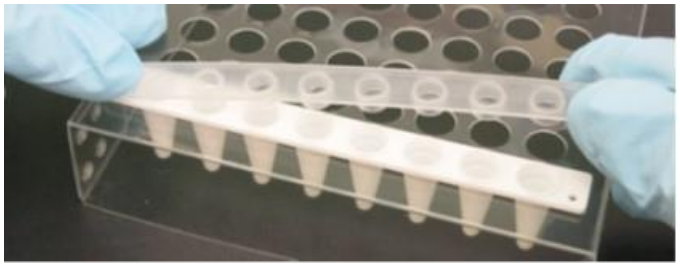
Reagent Preparation (2) (When BioRad PCR is used)

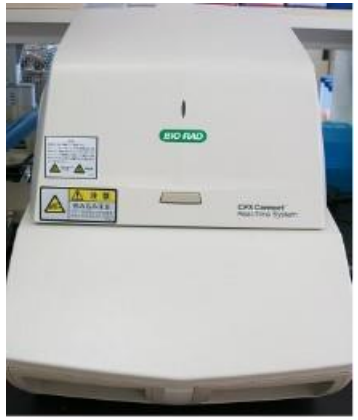
3) Peel off the film from test strip A and move all the dissolved sample in test strip B into test strip A, and pipet gently for 20 times to dissolve the solidified reagent.





4) Attach flat cap to test strip A, and detect by real-time PCR.

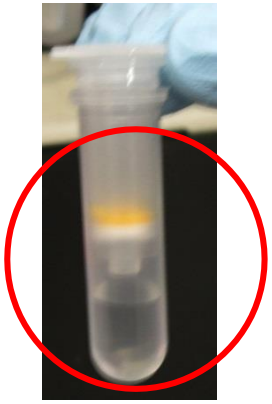




* When detection is performed using a transparent tube, first add the sample to test strip A, dissolve, and then move it into test strip B.

Precautions for extraction

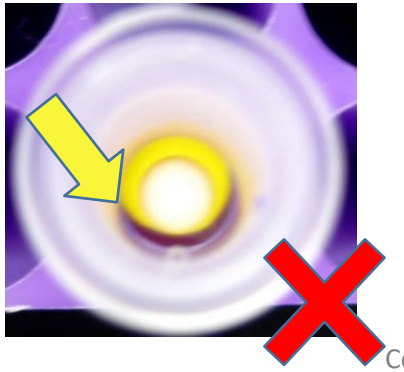
1) When performing the cleanup steps 4) through 7) of the extraction procedure using **QIAamp UCP MinElute Column,** be careful not to let the eluted sample in the collection tube contact the column.

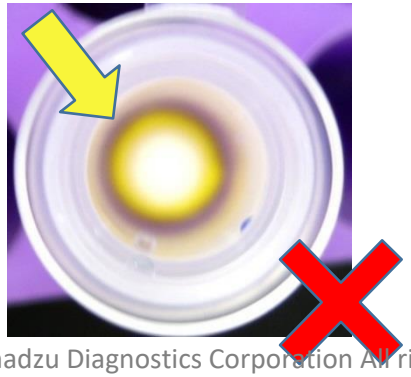


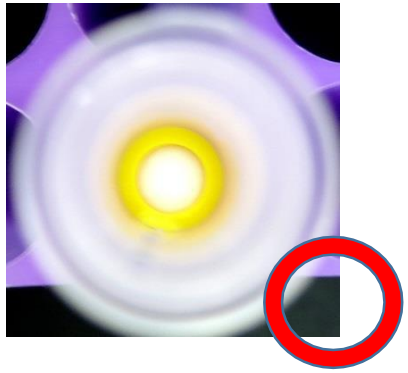
Operate carefully so that the liquid in the collection tube will not touch the bottom end of the column.

2) When eluting a sample using **Buffer AUE,** any remaining **Buffer AUW2** in the column will interfere with PCR**.** Check for any remaining Buffer and if there is, remove it by pipetting, etc. without damaging the filter.

Note) The liquid in the picture below is colored in blue for better visibility but actual liquid is transparent.







Disposal of strips after measurement

The test strips after PCR have to be disposed of with the caps on and sealed in a bag, etc. following the rules of the relevant facility.
If PCR product scatters in the work environment, it can cause contamination or false-positive result.
Make sure to dispose of the test strips used for PCR with their caps on.