



Protocol Tip

Bacteria DNA

1-1 Tips for Pre-treatment of specimens

- Dilute the sample by saline if it is thick.
- In the case of the sputum without viscosity, NALC-NaOH treatment step is NOT needed.

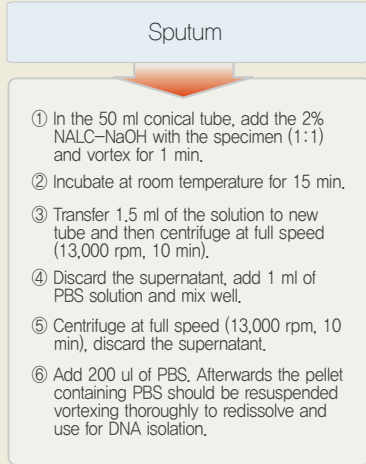
1-1 Pre-treatment of specimens

Nasopharyngeal aspirates, Nasopharyngeal swabs, Bronchoalveolar lavage

- ① Transfer 1.2 ml of the sample to 1.5 ml microcentrifuge tube.
- ② Centrifuge at full speed (13,000 rpm, 2-5 min).
- ③ Discard the supernatant leaving a small amount (200 ul) of the supernatant and pellet. Afterwards the pellet containing supernatant should be resuspended vortexing thoroughly to redissolve and use for DNA isolation.

1-2 Tips for Isolation of nucleic acids

- Always wear latex gloves while handling reagents and samples.
- Add 25 ml of 96-100% ethanol to a bottle of Buffer AW1 concentrate before using Buffer AW1 for the first time.
- Add 30 ml of 96-100% ethanol to a bottle of Buffer AW2 concentrate before using Buffer AW2 for the first time.
- For dissolving QIAGEN Protease, add 1.4 ml of Buffer AVE to the vial of lyophilized QIAGEN Protease.
- For dissolving carrier RNA, add 310 ul Buffer AVE to the tube lyophilized carrier RNA and then dissolved carrier RNA added to Buffer AL according to instructions (See Handbook p.17).
- Sufficiently vortex before adding specimen. When the specimen is still viscous, cool down its temperature or add cold PBS.
- Keep the isolated DNA at -20°C or on ice.



1-2 Isolation of nucleic acids (QIAamp MinElute Virus Spin Kit, QIAGEN)

25 ul QIAGEN Protease + 200µl Specimen + 200µl Buffer AL

Pulse-vortexing for 15 s and then incubate at 56°C for 15 mins.

Add 250µl of ethanol.

Pulse-vortexing for 15 s and then incubate at room temperature for 5 mins.

Apply all of the lysate onto the column and then centrifuge (8000 rpm, 1 min).

Place the column in a clean 2 ml collection tube.

Add 500µl of Buffer AW1 and then centrifuge (8000 rpm, 1min).

Place the column in a clean 2 ml collection tube.

Add 500µl of Buffer AW2 and then centrifuge (8000 rpm, 1min).

Place the column in a clean 2 ml collection tube.

Add 500µl of ethanol and then centrifuge (8000 rpm, 1min).

Place the column in a clean 2 ml collection tube.

Centrifuge at full speed (14,000 rpm, 3mins) to dry the membrane completely.

Place the column in a clean 1.5 ml microcentrifuge tubes.

Apply 40µl of Buffer AVE to the center of the membrane.

Incubate at room temperature for 2 mins.

Centrifuge at full speed (14,000 rpm, 1min).

User Quick Protocol

2 PCR step

① Add the following reagents to a PCR tube (on ice).

DNA	3
5X PB ACE PM	4
8-Mop solution	3
2X Multiplex Master Mix	10
Total Volume	20

No. of specimen	Mixture for different no. of specimen (unit: µl)									
	2	3	4	5	6	7	8	9	10	
	8	12	16	20	24	28	32	36	40	
	6	9	12	15	18	21	24	27	30	
	20	30	40	50	60	70	80	90	100	

② Gently mix the reactions without creating bubbles.

③ Place the tube in a preheated (94°C) thermal cycler.

④ Immediately commence the PCR reaction using the following program.

Step	Cycle	Temperature	Time
1	1	94°C	15 min
2-1		94°C	0.5 min
2-2	40	60°C	1.5 min
2-3		72°C	1.5 min
3	1	72°C	10 min

⑤ Electrophorese 2 ul of the PCR products on ScreenTape System or 5 ul of the PCR products on a 2 % agarose gel containing Et-Br.

2 Tips for PCR step

- Centrifuge any reagents stored at -20°C after melting them completely.
- If bands do not appear clearly on 2% agarose gel, you may try 2.5% gel for band separation. (However, the gel running time may take longer.)

Amplicon Size Information

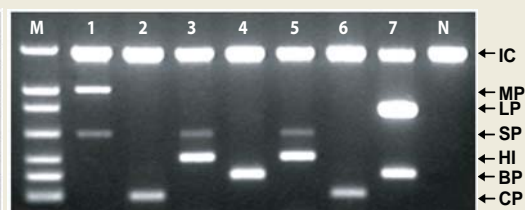
PneumoBacter ACE Detection	Size (bp)
	Agarose gel
Internal control	900
Mycoplasma pneumoniae	583
Legionella pneumophila	472
Streptococcus pneumoniae	350
Haemophilus influenzae	257
Bordetella pertussis	200
Chlamydophila pneumoniae	146

Application to clinical samples

ScreenTape® system (Lab901 Limited)

Lane	L9	L10	L11	L12	L13	L14	L15	L16								
Sample ID	1	2	3	4	5	6	7	N								
Internal Control	+	97	+	98	+	96	+	97	+	90	+	94	+	68	+	99
M. pneumoniae	+	48	-	-	-	-	-	-	-	-	-	-	-	-	-	-
L. pneumophila	-	-	-	-	-	-	-	-	-	-	-	-	+	100	-	-
S. pneumoniae	+	26	-	+	18	-	+	11	-	-	-	-	-	-	-	-
H. influenzae	-	-	-	+	96	-	+	87	-	-	-	-	-	-	-	-
B. pertussis	-	-	-	-	+	95	-	-	-	-	-	+	94	-	-	-
C. pneumoniae	-	-	+	90	-	-	-	-	-	+	76	-	-	-	-	-
Unidentified	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Agarose gel



M: PB ACE Marker, 1~7: Clinical samples, N: Negative control



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Ordering information

Cat. No.	Product	Size
Seeplex® Respiratory Pathogen Detection		
RV1211	Seeplex® RV12 Detection	50 rxns
RV2210	Seeplex® RV6 Detection	50 rxns
RV3210	Seeplex® RV7 Detection	50 rxns
RV6C00Y	Seeplex® RV12 ACE Detection	50 rxns
RV6550Y	Seeplex® RV5 ACE Screening	50 rxns
EV6100Y	Seeplex® Enterovirus ACE Detection	50 rxns
RP5J10Y	Seeplex® RV/PB18 ASE Detection	50 rxns
PB1610Y	Seeplex® PneumoBacter ACE Detection	50 rxns
Accessory products for Seeplex® series		
SG1300	cDNA synthesis kit	100 rxns
ScreenTape® System		
ST007	TapeStation	EA
ST214	ScreenTape® Loading tips (16 lane tape)	Pack (3,840 tips)