

RESPIRATORY VIRUS DETECTION KIT

Clinical trial version 1.0 (PCR only)

1 Protocol for Respiratory Virus Detection Kit

1.1 Reverse Transcription (not included in this kit)

1. Add the following reagents to a RT tube on ice.

α μ l	Total RNA (0.5 μ g)
2 μ l	10 μ M random Hexamer
7.5- α μ l	DEPC-treated water
9.5 μ l	

Note: Mix the reagents by tapping or pipetting.

Note: A wide range of patient RNA amount gives out good result. You may ignore the RNA concentration and use the maximum volume of 7.5 μ l. But we recommend 0.5 μ g of patient RNA.

2. Incubate the tube at 80°C for 3 min.
3. Chill the tube on ice for 2 min and spin the tube briefly.
4. Add the following reagents to the tube from step 3.

4 μ l	5X RT buffer
5 μ l	2 mM dNTP
0.5 μ l	RNase inhibitor (40 u/ μ l)
1 μ l	Reverse Transcriptase (200 u/ μ l)
20 μ l	Total volume

5. Incubate the tube at 37°C for 90 min.
6. Heat the tube at 94°C for 2 min.
7. Chill the tube on ice for 2 min and spin the tube briefly.

Note: Store all cDNA samples at -20°C until ready for use.

1.2 Respiratory Virus Detection PCR

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

3 μ l	First-strand cDNA (~10 ng)
4 μ l	5X SRVD-1 or 5X SRVD-2 (mixture of primers)
3 μ l	Distilled water
10 μ l	2X RV Master Mix
20 μ l	Total volume

Note: Depending on the samples, different amounts (1 - 5 μ l) of firststrand cDNA can be used as templates for PCR.

2. Place the tube in a preheated (94°C) thermal cycler (optional).
3. Immediately commence the PCR reaction using the following program.

Segment	No. of cycles	Temperature	Duration
1	1	94°C	15 min
2	40	94°C	30 sec
		60°C	1.5 min
		72°C	1.5 min
3	1	72°C	10 min

Note: We recommend the GeneAmp PCR System 9700 of Applied Biosystems that has a heated lid. It will be necessary to determine the optimal temperature for your individual thermocycler.

4. Electrophorese 5 μ l of the PCR products on a 2% agarose gel containing EtBr.

Note: If the band intensity of your sample is very weak, the intensity can be increased by raising the amount of first-strand cDNA or SRVD primer in the PCR mixture or by increasing the number of PCR cycles up to 45 cycles.

1.3 Positive Control

Note: RV control contains a mixture of 11 virus control DNAs. For control PCR, use 2 µl of RV control instead of First-strand cDNA in the PCR reaction mixture like the following.

2 µl	RV control
2 µl	5X SRVD-1 or 5X SRVD-2
6 µl	Distilled water
10 µl	2X RV Master Mix
20 µl	Total volume

Note: For the other PCR conditions, refer 1.2 Respiratory Virus Detection PCR part.

Note: Electrophorese 2 µl of the control PCR products on a 2% agarose gel containing EtBr as a standard size marker and a control for PCR reaction.

Note: The amplicon sizes using 5X SRVD-1 or 5X SRVD-2 as primers are indicated at the following table.

SRVD-1 set	Amplicon length (bp)	SRVD-2 set	Amplicon length (bp)
Human adenovirus	534	Influenza A virus*	513
Human metapneumovirus	423	Influenza B virus	455
Human coronavirus 229E / NL63	375	Human respiratory syncytial virus B	391
Human parainfluenzavirus 1	324	<i>Rhinovirus</i>	<i>Not in this version</i>
Human parainfluenzavirus 2	264	Human respiratory syncytial virus A	273
Human parainfluenzavirus 3	219	Human coronavirus OC43	231

* This primer set can also detect influenza A(H5N1) virus causing highly pathogenic avian influenza.

1.4 Negative Control

Use 2 µl of water instead of RV control in the PCR mixture.

Note: To prevent contamination from PCR products, cotton tip is recommended. Please be careful not to contaminate the SRVD primer sets with PCR products through pipet. It is recommended that exclusive use of pipet for PCR products or pipet for primers.

2 Expected Results

2.1 Positive control PCR

As a positive control, viral RNA from viral culture soup was used in RT-PCR which is conducted with the SRVD primer mix in accordance with the instructions given in this User Manual. Below are examples of resulting data shown by agarose gel photographs. These agarose gels show the typical results that are generated by such experiments.

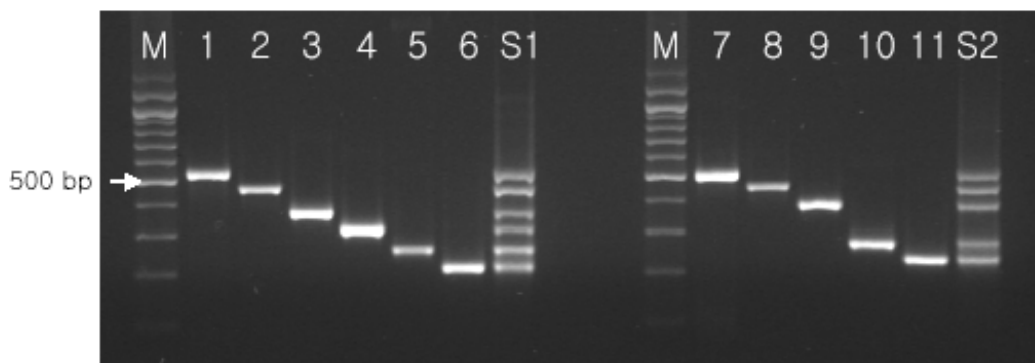
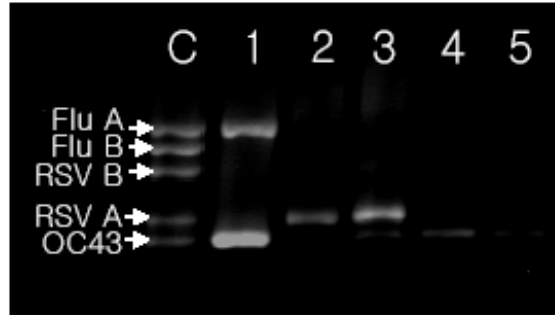


Fig. 1 Multiplex-PCR for the positive control. M;100 bp ladder (Seegene Cat. No. M0100), 1;Human adenovirus, 2;Human metapneumovirus, 3;Human coronavirus 229E, 4;Human parainfluenza virus 1, 5;Human parainfluenza virus 2, 6;Human parainfluenza virus 3, S1;SRVD-1 set using RV control as the template, 7;Influenza A virus, 8;Influenza B virus, 9;Human respiratory syncytial virus B, 10;Human respiratory syncytial Virus A, 11;Human coronavirus OC43, S2;SRVD-2 set using RV control as the template.

2.2 Application to clinical samples

A. SRVD-1 set



B. SRVD-2 set

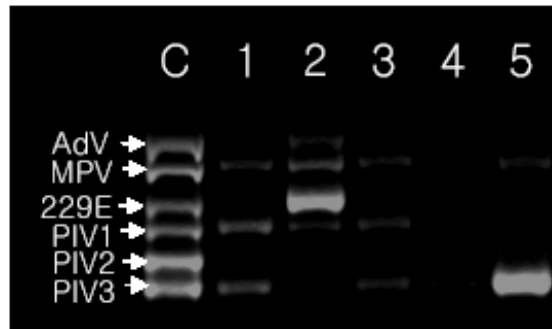


Fig. 2 Detection of respiratory viruses from clinical samples.

C; Positive control, **1;**patient 1, **2;**patient 2, **3;**patient 3, **4;**patient 4, **5;**patient 5, **AdV;**adenovirus, **MPV;**metapneumovirus, **229E;**coronavirus 229E / NL63, **PIV1;**parainfluenza virus 1, **PIV2;**parainfluenza virus 2, **PIV3;**parainfluenza virus 3, **Flu A;**influenza A virus, **Flu B;**influenza B virus, **RSV B;**respiratory syncytial virus B, **RSV A;**respiratory syncytial virus A, **OC43;**coronavirus OC43.

3 Components

3.1 List of components

1. Cap number 1, **5X SRVD-1**, 140 µl
A Mixture of 12 primers for 6 viruses
2. Cap number 2, **5X SRVD-2**, 140 µl
A Mixture of 10 primers for 5 viruses
3. Cap number 3, **RV control**, 100 µl
A Mixture of 11 viral clones
4. **2X RV Master Mix**, 500 µl X 2 tube
Optimized for Respiratory Virus Detection Kit
Contains DNA polymerase and dNTP in 2X buffer
5. **Manual**
For Respiratory Virus Detection Kit
(Clinical trial version 1.0)

3.2 Storage Conditions

Store all reagents below -20°C except the RV Master Mix.

In case of the RV Master Mix, 4°C for short-term storage and -20°C for long-term storage are recommended. Avoid repetitive thawing as it may decrease the activity of the Master Mix.

3.3 Reagents and Equipments to be Supplied by the User

RNase-free H ₂ O	Reverse transcriptase	2 mM dNTP
RNase inhibitor	Thermal cycler	Micro-centrifuge
Random Hexamer		

4 Ordering information

Cat. No.	Products	Size
RV1210	Respiratory Virus Detection Kit (PCR kit)	(50+20)X2 rxns