

Protocol: Real-Time PCR Kit for Respiratory Syncytial Virus A/B

A. Introduction

The RSV A/B r-gene™ Primers/Probe kit from ARGENE S.A. is designed to amplify RSV A and RSV B viruses in a duplex reaction using TaqMan® probes. Target sequences are located in a gene coding for a nucleocapsid (N) protein, and correspond to an amplicon of 185 bp. Both RSV A and RSV B probes emit at wavelengths detectable by the single fluorescence channel of the Spartan DX™. This allows the instrument to identify a sample as RSV positive, although it does not differentiate between RSV A and B.

This protocol takes about 20 min for PCR preparation, 30 min for reverse transcription, and 60 min to perform real-time PCR.

B. Equipment

- Spartan DX instrument
- Microcentrifuge
- Vortex
- Ice bucket or cold block
- Pipettes

C. Materials

- 0.2 ml flat-cap PCR tubes (Fisher Scientific, Cat. No.08-408-214)
- Filtered pipette tips
- PCR-grade Mineral Oil (Biotools, Cat. No. 20.032)
- RSV A/B r-gene™ Primers/Probes kit (ARGENE, Cat. No. 71-007)
- OneStep RT-PCR Enzyme Mix (QIAGEN, Cat. No. 210210/210212)
- Viral RNA Mini Kit (QIAGEN, Cat No.52904)
- Sterile water (DNase-and RNase-free)

D. Preparation

1. Turn on Spartan DX instrument and leave to warm up for a minimum of 10 min
2. Set up thermal cycling program as per Table 1
3. We recommend the use of Viral RNA Mini Kit (QIAGEN) for RNA extraction

Step	Temperature	Time	Cycles
Reverse Transcription	50°C	30 min	1
Initial denaturation	95°C	15 min	1
Denaturation	95°C	30 s	50
Annealing/extension	45°C	30 s	50

Table 1. Cycling parameters.

E. Protocol

1. Prepare a master mix for RSV, as per Table 2
2. To test one unknown sample, we recommend setting up reactions as follows:
 - Tube 1: 15 µl of RSV master mix
 - Tube 2: 15 µl of RSV master mix
 - Tube 3: 15 µl of RSV master mix

3. In a separate lab area, add the following:
 - Tube 1: 5 µl of known RSV-positive RNA (positive control)
 - Tube 2: 5 µl of sterile water (negative control)
 - Tube 3: 5 µl of extracted RNA (sample in question)
4. Mix and spin down reactions
5. Overlay reaction mixture with 15 µl of mineral oil
6. Spin down reactions again
7. Insert samples into Spartan DX instrument and start your run
8. Representative real-time PCR result is shown in Figure 1

Reagent	RSV Master Mix	
	Reaction Formulation	Volume
ARGENE RSV A/B Mix (2X)	10 µl x (____+0.5*) Sample #	
QIAGEN One step Enzyme Mix (0.5U)	0.5 µl x (____+0.5*) Sample #	
Sterile water	4.5 µl x (____+0.5*) Sample #	
Total volume of master mix	15 µl/reaction	____ µl

* Recommended volume correction factor for pipetting error.

Table 2. Components of PCR amplification mixture.

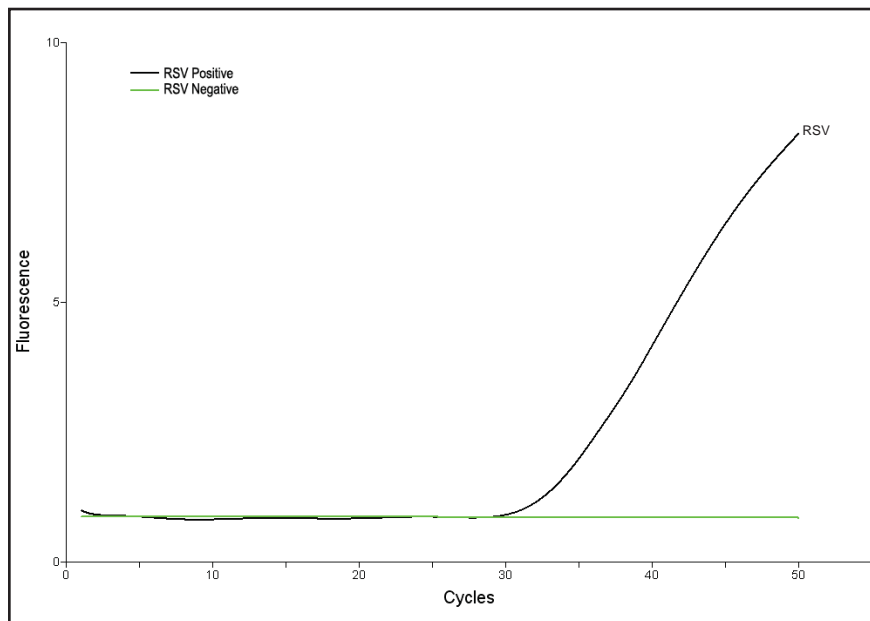


Figure 1. Real-time graph of RSV results.

Disclaimer

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