PROMISE RSV-LabNet Protocol Library

PCR - RSV A/B

Updated 18 January 2023

This protocol has been made available by:

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This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement 101034339. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. This document reflects the author's view and that neither IMI nor the European Union, EFPIA are responsible for any use that may be made of the information contained therein.

1. Reagents

RNA Extraction
Using the Maelstron 9600 automated extraction platform from Taiwan Advanced Nanotech (TANBead) with the TANBead Nucleic Acid Extraction Kit (Catalog Number: 301224).

Lightcycler PCR or CFX Series PCR
PCR reactions should be performed on Lightcycler 480 or Bio-Rad CFXConnect or CFX96 with Thermo Fisher Scientific™ (Invitrogen™) SuperScript III One-Step RT-PCR System with Platinum Taq DNA Polymerase Master Mix (Thermo Fisher Scientific Catalog Number: 12574-026).

Primer Sets
RSV-A & B
Primer 1: RSV-K forward: 5' GGA AAC ATA CGT GAA CAA GCT TCA 3'
Primer 2a: RSV-K A rev: 5' CAT CGT CTT TTT CTA AGA CAT TGT ATT GA 3'
Primer 2b: RSV-K B rev: 5' TCA TCA TCT TTT TCT AGA ACA TTG TAC TGA 3'
RSV Probe: 6FAM-TGT GTA TGT GGA GCC TT-MGB (ABI)

Primers and Probes Information

RSVA/B mix	Label	Filter (nm)	pmol in PCR-mix	Subset
PRIMER 1: RSV-K fwd			**20 pmol/ul**	**RSV-A/B**
Primer 2a: RSV-K A rev			**10 pmol/ul**	
Primer 2b: RSV-K B rev			**10 pmol/ul**	
RSV Probe	6-Fam - MGB	465-510	**4 pmol/ul**	

Target	Sequence	Filter (nm)	Subset name
RSVA/B	6-Fam – MGB	465-510	RSVA/B

2. Procedure

Preparation
1. Make appropriate dilutions of positive controls.
2. Isolate RNA by TANBead Maelstrom 9600 platform and TANBead Nucleic Acid Extraction Kit (Catalog Number: 301224)
3. Make PCR-mix for RSVA/B (reagents lab).

PCR-Mix	conc.	[end-conc.]	µl
PCR Grade Water			5.1
MgSO4			0.4
2x Reaction Mix			12.5
RSV-primer/probe mix			1.0
SIII/Taq Enzyme Mix			1.0
Total volume			**20.0**

Sample Addition, Reverse Transcription, and PCR
1. Aliquot 20 ul portions of PCR-mix in 96-well plate according to plate layout template
2. Add 5 uL RNA to 20 ul PCR-mix
3. Seal the 96-well plate

4. Centrifuge briefly
5. Keep plate at 4ºC if you can't run directly
6. Run PCR Program on LightCycler 480 or CFXConnect or CFX96
7. Analyse results

PCR-program LightCycler 480 or CFXConnect or CFX96:

Detection Format:	**Multi Color Hydrolysis Probe**				
PCR Program	**Segment Number**	**Temp (ºC)**	**Hold Time (sec.)**	**Slope (ºC/sec.)**	**Acquisition Mode**
Reverse Transcription	1	50	1200		
Denaturation	1	94	180		
Amplification (Cycles: 45)	1 2	94 58	15 30		None Single
Cooling	1	40	30		None

Positive Control Information*

Control	**Name**
Positive Control	**Current strains from own repository**

***Note –** The positive control material used at Medical University of Vienna are cultured strains from the biobank (Center of Virology, Medical University of Vienna), diluted in DMEM with a target Ct value of 27.

Additional Note(s)

Protocol adapted from: Applicability of a Real-Time Quantitative PCR Assay for Diagnosis of Respiratory Syncytial Virus Infection in Immunocompromised Adults (L.J.R. van Elden et al.) – J. Clin. Microbiol. 2003