PROMISE RSV-LabNet Protocol Library

PCR - RSV A/B

Updated 18 January 2023

This protocol has been made available by:

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1. Reagents

RNA Extraction

MagNa Pure 96 System (Roche Material Number: 065 410 890 01) using MagNA Pure 96 DNA and Viral Nucleic Acid Small Volume Kit (Roche Catalog Number: 065 435 880 01)

ABI7500 Real Time PCR Machine (Applied Biosystems)

PCR reactions are performed on either a TaqMan 7500 with Superscript III platinum one-step quantitative RT-PCR (Cat Number 11732-088)

Primer Sets

RSV-A

RSVA1	AGA TCA ACT TCT GTC ATC CAG CAA
RSVA2	TTC TGC ACA TCA TAA TTA GGA G
RSVA-Probe	*5'-CY5* CAC CAT CCA ACG GAG CAC AGG AGA T *3'-BHQ2*

RSV-B

RSVB1	AAG ATG CAA ATC ATA AAT TCA CAG GA
RSVB2	TGA TAT CCA GCA TCT TTA AGT ATC TTT ATA GTG
RSVB-Probe	*5'-FAM* TTC CCT TCC TAA CCT GGA CAT AGC ATA TAA CAT ACC *3'-BHQ1*

Primers and Probes Information

RSV-A/B mix	Label	Filter (nm)	pmol in PCR-mix	Subset
RSVA1			**0.8uM**	
RSVA2			**0.8uM**	**RSVA**
RSVA-Probe	CY5-BHQ2	672-712	**0.4uM**	
RSVB1			**0.8uM**	
RSVB2			**0.8uM**	**RSVB**
RSVB-Probe	FAM-BHQ1	470-490	**0.4uM**	

Target	Sequence	Filter (nm)	Subset name
RSVA	CY5-BHQ2	672-712	RSVA
RSVB	FAM-BHQ1	470-490	RSVB

2. Procedure

Preparation

1. Make appropriate dilutions of positive controls.
2. Isolate RNA by MagNA Pure 96 DNA and Viral Nucleic Acid Small Volume Kit (Roche Catalog Number: 065 435 880 01)
3. Make PCR-mix for RSVA, RSVB (reagents lab)

TaqMan 7500 PCR-Mix	μl
Superscipt III platinum one-step reaction buffer	12.5
Superscipt III platinum one-step ezyme	0.5
RSV-A/B Detection mix	1.5
ROX 1:10 dilution	0.5
PCR Grade Water	5.0
Total volume	**20.0**

96-well 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. Run PCR Program on TaqMan 7500 instrument
6. Analyze results

Notes – None

PCR-program TaqMan 7500:

	Temperature	Time	Number of Cycles
RT	50ºC	30 minutes	1
Denature	95ºC	2 minutes	1
Cycle	95ºC	15 seconds	40
	55ºC	30 seconds	

Positive Control Information*

Control	Name
Positive Control	**Positive control material from Zeptometrix Diluted 1:5**

*Note – The positive control material used at NVRL are strains provided by Zeptometrix®

Additional Note(s)

Assay validation report is available if requested. The PCR has been tested in the following External Quality Assessments: NEQAS molecular detection of respiratory viruses, ECDC ERLI-Net Influenza panel 2018, QCMD Influenza haemagglutinin typing, QCMD respiratory 1 EQA pilot, WHO EQA programme for detection of Influenza Type A by PCR.