



**PROMISE**  
Preparing for RSV Immunisation  
and Surveillance in Europe

## **PROMISE RSV-LabNet Protocol Library**

### **PCR - RSV A/B**

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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	RSVA
RSVA2			0.8uM	
RSVA-Probe	CY5-BHQ2	672-712	0.4uM	
RSVB1			0.8uM	RSVB
RSVB2			0.8uM	
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)

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

### **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 Denature	50°C	30 minutes	1
	95°C	2 minutes	1
Cycle	95°C	15 seconds	40
	55°C	30 seconds	

### **Positive Control Information\***

Control	Name
Positive Control	Positive control material from Zeptomatrix Diluted 1:5

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

### **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.