



PROMISE
Preparing for RSV Immunisation
and Surveillance in Europe

PROMISE RSV-LabNet Protocol Library

PCR - RSV A/B

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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 with the total Nucleic Acid Kit (Roche Catalog Number: 06374891001).

Lightcycler PCR

PCR reactions should be performed on Lightcycler 480 with TaqMan™ Fast Virus 1-Step Master Mix (ThermoFisher Scientific Catalog Number: 444 443 2). **Note:** Erasmus Medical Center includes uracil DNA glycosylase (UDG) which is optional when ordering Fast Virus 1-Step Master Mix from ThermoFisher. If interested, contact your Life Technologies representative according to the user guide.

Primer Sets

RSV-A

RSVA-fwd AGA TCA ACT TCT GTC ATC CAG CAA
 RSVA-rev TTC TGC ACA TCA TAA TTA GGA GTA TCA AT
 RSVA-probe 5'-*DragonFly* – CAC CAT CCA ACG GAG CAC AGG AGA T 3'-*BHQ2*

RSV-B

RSV-B-TM-fwd TTC CTA ACT TCT CAA GTG TGG TCC TA
 RSV-B-TM-rev CTG GTT TCT TGG CGT ACC TCT ATA C
 RSV-B-TM-probe 5'-*DragonFly* TCC CAT TAT GCC TAG ACC TGC TGC ATT G 3'-*BHQ2*

Primers and Probes Information

RSVA/B mix	Label	Filter (Nm)	pmol in PCR-mix	Subset
RSVA-fwd			45 pmol/ul	RSVA
RSVA-rev			30 pmol/ul	
RSVA-probe	DragonFly-BHQ2	554-576	5 pmol/ul	
RSVB-fwd			45 pmol/ul	RSVB
RSVB-rev			15 pmol/ul	
RSVB-probe	DragonFly-BHQ2	554-576	5 pmol/ul	

Target	Sequence	Filter (nm)	Subset name
RSVA	DragonFly-BHQ2	554-576	RSVA
RSVB	DragonFly-BHQ2	554-576	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/B (reagents lab)

PCR-Mix	conc.	[end-conc.]	µl
4x Taqman Fast Virus MM			5.0
RSV-primer/probe mix			0.4
PCR Grade Water			6.6
Total volume			12.0

Sample Addition, Reverse Transcription, and PCR

1. Aliquot 12 ul portions of PCR-mix in 96-well plate according to plate layout template
2. Add 8 uL RNA to 12 ul PCR-mix
3. Seal the 96-well plate
4. Centrifuge briefly
5. Keep plate at 4°C if you can't run directly
6. Run PCR Program on LightCycler 480
7. Analyse results

PCR-program LightCycler 480:

Detection Format:	Multi Color Hydrolysis Probe				
PCR Program	Segment Number	Temp (°C)	Hold Time (sec.)	Slope (°C/sec.)	Acquisition Mode
Reverse Transcription	1	50	300		
Denaturation	1	95	20		
Amplification (Cycles: 45)	1	95	3		None
	2	60	30		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 Erasmus Medical Center are strains from the EMC repository, cultured at Erasmus Medical Center, diluted in DMEM with a target Ct value of 26. If positive control material is needed, please request through [EVAg portal](#).

Additional Note(s)

Erasmus Medical Center Viroscience Department uses the Hologic Panther Fusion™ assay (FluA/FluB/RSV - Part # PRD-04328) for routine diagnostics (<https://pubmed.ncbi.nlm.nih.gov/31869661/>). The above in-house assay is a “back-up” assay.