

Erasmus MC

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Protocol for Influenza RT-PCR Viroscience ErasmusMC

Primers and Probes

Detection:

Influenza A-Matrix:

infa_fwd	5'-CTTCTRACCGAGGTCGAAACGTA	45 pmol/reactie	
infa_probe1	5'-TCAGGCCCCCTCAAAGCCGAGA	5 pmol/reactie	FAM-BHQ1
infa_probe2	5'-TCAGGCCCCCTCAAAGCCGAAA	5 pmol/reactie	FAM-BHQ1
infa_rev	5'-TCTTGTCTTTAGCCAYTCCATGAG	45 pmol/reactie	

Influenza B-NS

infb-NSfwdB	5'-GRACAACATGACCACAACACAAAT	30 pmol/reactie	
infb-NSrevB	5'-CACTCCARAATTCCTGCTTCAA	45 pmol/reactie	
infb-NSprobeB	5'-CGGGAGCAACCAATGCCACCATAAA	5 pmol/reactie	DFO ¹ -BHQ2

Typing of Influenza A:

H1

H1-fwd1	5'-CCAAAGTATGTCAGGAGTGCAAAAT	30 pmol/reactie	
H1-fwd2	5'-CCAAAGTATGTCAGGAGTACAAAAT	30 pmol/reactie	
H1-rev	5'-CCTTCAATGAAACCGGCAAT	30 pmol/reactie	
H1-probe	5'-TGGTTACAGGACTAAGGAACATCCCATCCA	5 pmol/reactie	FAM-BHQ1

H1 pd2009

MexFlu-H1-fpr	5'-GGAAAGAAATGCTGGATCTGGTA	30 pmol/reactie	
MexFlu-H1-rpr	5'-ATGGGAGGCTGGTGTATAGC	30 pmol/reactie	
MexFlu-H1-prb	5'-TGCAATACAACCTTGTCAGACACCCAAGGG	10 pmol/reactie	DFO-BHQ2

H3

H3HA-fwd	5'-GCAACTGTTACCCATTATGATGT	45 pmol/reactie	
H3HA-rev	5'-CCAGTCCAATTGAAGCTTTC	45 pmol/reactie	
H3HA-probe	5'-CTTAGGTCAGTGTGCMTCA	10 pmol/reactie	FAM-BHQ1

¹ DFO: DragonFly Orange

PCR Mix

Taqman Fast Virus one step Mastermix (4X FVMM; Thermofisher)

FVMM	5 ul
Primers/probes	0.4 ul
PCR grade water	6.6 ul
RNA template	8.0 ul
Total	20 ul

Amplification protocol (Roche LightCycler480)

2 min	50 °C	
20 sec	95 °C	
3 sec 30 sec	95 °C 60 °C	45 cycli