

Anlage 1 zu: NRL-AI PCR_40	Real-time RT-PCR zum Nachweis von Influenza A Viren des Subtyps H5 und Pathotyps HP (Methode H5-2344b_DE)	Version 01
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Lab-No:	RNA-Extraction:		RT-qPCR:		
	Date:	Processor:	Date:	Time:	Processor:
Cycler:			Memory location:		
Notes:					

Mastermix AgPath-ID™ One-Step RT-PCR Reagents, Ambion-Applied Biosystems	H5-2344b_DE-Mix 1	
	1 x	16x
RNase free water	4.5 µl	72
2x RT-PCR Buffer	12.5 µl	200
RT-PCR Enzyme Mix	1.0 µl	16
H5-HP - Primer-Probe Mix	2.0 µl	32
total volume Mastermix:	20.0 µl	
RNA-Template (Samples, PC, NTC)	5.0 µl	

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Cycler-Program:	RT	10 min	45°C	45 cycles
# detection: FAM during annealing step	Activation Taq	10 min	95°C	
	Denaturation	15 sec	95°C	
	Annealing	20 sec	56°C	
	Elongation	30 sec	72°C	

Generation of 200 µl H5-2344b DE-Primer-Probe-Mix:

Volume	Oligo (100pmol/µl)	Sequence (5` - 3`)
20.0 µl	2344b_H5_F3	ccttgcgactgggctcag
20.0 µl	2344b_H5_R2	atcaaccattccctgcca
5 µl	2344b_DE_Sonde	FAM-AGA AGA AAR AGA GGG CTG TTT GGG GCT-BHQ-1
155 µl	0,1 x TE (pH 8,0)	

PC= H5 (HP) N8 [AR8444/16]

valide: