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| Friedrich-Loeffler-Institut Prof. Dr. Timm Harder | Real-time RT-PCR for AIV subtyping - RT-qPCR_N8_Mix 1 - | created by: Prof. Dr. Timm Harder on: 11.11.2014 Version 1 |
|---|--|---|

| Lab-No: | RNA-Extraction: | | RT-qPCR: | | |
|---------|-----------------|------------|------------------|-------|------------|
| | Date: | Processor: | Date: | Time: | Processor: |
| | | | | | |
| Cycler: | | | Memory location: | | |
| Notes: | | | | | |

| Mastermix AgPath-ID™ One-Step RT-PCR Reagents; Art-Nr: #4387424 oder Art-Nr: #4387391, Ambion-Applied Biosystems | N8-Mix 1 | |
|--|----------|-----|
| | | 1 x |
| RNase free water | 4.5 µl | |
| 2x RT-PCR Buffer | 12.5 µl | |
| RT-PCR Enzyme Mix | 1.0 µl | |
| N8-Primer-Probe Mix 1 | 2.0 µl | |
| | | |
| total volume Mastermix: | 20.0 µl | |

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|--|------|
| RNA-Template (Samples, RNA isolation controls, PC, NTC) | 5 µl |
|--|------|

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

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

The N8-Mix-1 assay amplifies a 128 bp fragment of the NA genome segment of N8 influenza A viruses.

Generation of 200 µl N8-Primer-Probe-Mix 1:

| Volume | Primer name | Sequence (5' - 3') |
|----------------------------|------------------|---|
| 20 µl of 100 pmol/µl stock | IVA-N8-1296F | TCC ATG YTT TTG GGT TGA RAT GAT |
| 20 µl of 100 pmol/µl stock | IVA-N8-1423R | GCT CCA TCR TGC CAY GAC CA |
| 10 µl of 100 pmol/µl stock | IVA-N8-1354FAM | FAM-TCH AGY AGC TCC ATT GTR ATG TGT GGA GT-BHQ1 |
| 150 µl | 0.1x TE (pH 8.0) | |