**q-RT PCR Mastermix Recipe for WNV Surveillance Week 31 – 8.3.17**

Collect supplies:

1000 uL and 200 uL pipettes and tips, 15 uL conical for 7(27x) master, seven 1.7 mL tubes for 27X aliquots, two ice containers (1 for in and outside of BSC), and a vortex and centrifuge.

Prep hood with 70% ETOH and RNAase-away.

Collect reagents:

2X ITAQ, nfH20, F Primer (10 uM), R Primer (10 uM), FAM Probe (10 uM).

Put frozen reagents on ice to thaw.

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| --- | --- | --- | --- |
| Reagent | uL per reaction | uL per 27 reactions | (7)\*27x (ul) |
|  |  |  |  |
| 2X iTaq | 10 | 270 | 1890 |
| nfH20 | 2.5 | 67.5 | 472.5 |
| Forward 10 uM | 0.9 | 24.3 | 170.1 |
| Reverse 10 uM | 0.9 | 24.3 | 170.1 |
| FAM 10 uM | 0.45 | 12.15 | 85.05 |
|  |  |  |  |
| RNA | 5 | N/A | N/A |
| RT | 0.25 | 6.75 | N/A |

Vortex and spin down two mL of ITAQ, transfer to BSC (ETOH and RNase). Add 1890 uL to conical.

Add nfH20, add 472.5 uL.

Vortex and spin down both F and R primers, transfer to BSC (ETOH and RNase). Add 170.1 uL of each.

Vortex and spin down FAM probe, transfer to BSC (ETOH and RNase). Add 85.05 uL.

Vortex 50 uL conical for 10 seconds, ETOH/RNAse back into BSC on ice.

Set up seven 1.7 mL tubes on ice.

Aliquot 398.25 uL into each 1.7 uL tube. Store at -20 until ready for use.

Add 6.75 uL of RT immediately before use.