**q-RT PCR Mastermix Recipe for WNV Surveillance Week 33 – 8.17.17**

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, one ice containers, and a vortex and centrifuge.

Prep bench 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 | (4)\*27x (ul)+5% |
|  |  |  |  |
| 2X iTaq | 10 | 270 | 1134 |
| nfH20 | 2.5 | 67.5 | 283.5 |
| Forward 10 uM | 0.9 | 24.3 | 102.1 |
| Reverse 10 uM | 0.9 | 24.3 | 102.1 |
| FAM 10 uM | 0.45 | 12.15 | 51 |
|  |  |  |  |
| 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 **1134** uL to conical.

Add nfH20, add **283.5** uL.

Vortex and spin down both F and R primers, transfer to BSC (ETOH and RNase). Add **102.1** uL of each.

Vortex and spin down FAM probe, transfer to BSC (ETOH and RNase). Add **51** uL.

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

Set up **four** 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.

Note: Unlike previous weeks, this is being done outside of a BSC, at James’ recommendation. (The BSC is a reasonable precaution for huge batch preps, but not necessary when doing one plate at a time.)