**q-RT PCR Mastermix Recipe for WNV Surveillance (19 aliquots, fifteen 27 X and four 13x)**

Collect supplies:

1000 uL and 200 uL pipettes and tips, 15 uL conical for master, 1.7 mL tubes for 27X aliquots, ice container, vortex, and centrifuge.

(Perform large preps in BSC. Daily preps can be done at bench.)

Collect reagents:

EXPRESS qPCR Supermix, nfH20, F Primer (**10 uM**), R Primer (**10 uM**), FAM Probe (**10 uM**).

Thaw frozen reagents by hand and then store on ice.

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| --- | --- | --- | --- |
| Reagent | uL per reaction | uL per 27 reactions | [17]\*27x + 5% (uL) |
|  |  |  |  |
| EXPRESS qPCR Supermix A10312 | 10 | 270 | 4,819.5 |
| nfH20 | 1.5 | 40.5 | 722.9 |
| Forward 10 uM | 1 | 27 | 482 |
| Reverse 10 uM | 1 | 27 | 482 |
| FAM 10 uM | 0.5 | 13.5 | 241 |
|  |  |  |  |
| RNA | 5 | N/A | N/A |
| Superscript A10316 (RT) | 1 | 27 | N/A |

* Vortex and spin down Invitrogen **Supermix**. Add **4819.5** uL to conical.
* Add nfH20, add **722.9** uL.
* Vortex and spin down F and R primers. Add **482** uL of each.
* Vortex and spin down FAM probe. Add **241** uL.

Vortex 50 uL conical for 10 seconds.

Set up **nineteen** 1.7 mL tubes on ice.

Aliquot 378 uL into fifteen 1.7 uL tube. Label 27x.

Aliquot 182 uL into four 1.7 uL tube. Label 13x.

Store at -20 until ready for use.

Add 27 uL of Invitrogen **Superscript** (RT) to each 27x tube immediately before use.

(13 uL for 13x tubes.)