**Taqman qPCR Copy Number Detection of Zika Virus**

**MATERIALS**

Reagent; Company/Catalog Number:

1. TaqMan Universal PCR Master Mix; Applied Biosystems/REF 4304437
2. IDT primers/5’ FAM probe
3. Plasmid for standard [Capsid, prME or NS2b]
4. **Dilute plasmid to make standard**

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| **Plasmid** | **DNA (ng/µl)** | **Plasmid vol (µl) to make 1 ml 10^8 copies/µl** |
| pEF FI.prME | 538.5 | 1.17 µl |
| pEF FI.Capsid | 239.8 | 2.16 µl |
| pEF FI.NS2B/3 | 571 | 1.179 µl |

* 1. With the current batch of prME standard, 4.19 µL into 1 mL will give 10^8 copies/µl. If you dilute 4.19 µL of standard into 3 mL, you can then have 10^8 copies in 3 µL. You will want to add the same amount of sample as standard, so if you add 3 µL sample, add 3 µL standard, etc.
  2. Make serial 10-fold dilutions in water: 10^8, 10^7… I like to include a 50 (7^2) and 25 (5^2) in there as it gets closer to the limit of detection. I go to 10 or 1.

1. **Make Master Mix**
   1. Use TaqMan Universal PCR Master Mix containing AmpliTaq Gold DNA Polymerase
   2. Add 7 µl Master Mix per well using the E1-ClipTip Electronic Single Channel Pipette

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| **Reagent** | **For animal experiments or hard to detect samples** | **For standard cell culture experiments – optimized by Megan** |
| TaqMan Universal Master Mix | 5 µl | 5 µl |
| ZIKV FWD primer (500 nM) | 0.5 µl | 0.0625 µl |
| ZIKV REV primer (500 nM) | 0.5 µl | 0.0625 µl |
| ZIKV Probe (250 nM) | 0.25 µl | 0.03125 µl |
| Nuclease-free water | 0.75 µl | To 10 µl final vol |
| cDNA (I usually use 3, you can use 2.5 µl and then change water to 10 µl final vol | 3 µl | 2.5 µl |

1. **Add samples** (or do steps 2 and 3 in the order you prefer)
   1. cDNA samples:
      1. NHP samples (no dilution and use 3 µl of cDNA)
      2. Cell samples (400 ng; dilute 1:8) for approx. 50 ng input cDNA
   2. Add 3 µl cDNA sample in triplicate wells using the pink ClipTip pipette
   3. Seal plate using plate sealer and spin at 1000 rpm for 1 min
2. **Set up plate on ViiA™7 Real-Time PCR System** (Thermo Fisher Scientific)
   1. Reserve a 2 hr block of time for the qPCR cycler
   2. Select Taqman, standard curve, standard (i.e. not the “fast” option).
   3. Be sure to set your probe correctly from the get go. The ZIKV Capsid and ZIKV NS2b set has a FAM and NFQ-TMB quencher (ZIKV PRME set has a FAM no quencher). Set this up in the drop down by the target name.
   4. Assign your samples as you usually would for non-copy #
   5. **Define standards** by clicking on the “define standards” button. Choose “Let me select the wells” so that you can highlight where you want your standards to go and if you want them to be in columns or rows. Select “ROWS”
      1. # of points: 7
      2. # of replicates: 3
      3. Starting quantity: 10^8
      4. Serial factor: 10
      5. Enter 50, 25 and 10 individually
   6. Set a NTC (non-targeting control); aka water instead of sample. This will help set the threshold and tell you if there is any contamination. This sample should be negative.
   7. **Run method: 278882297**
      1. Reaction vol per well: 10 µl
      2. STEP 1 x 1 (Hold stage): [50˚C for 2:00 min, 95˚C for 10:00 min]
      3. STEP 2 x 40 (PCR stage): [95˚C for 0:15 sec, 60˚C for 1:00 min]
      4. Run 52 cycles
   8. At the end of the run, save file, hit “Analysis” button and export Excel file
   9. Analysis: cut-off of Ct 38 for NHP samples

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| **Assay** | **Primer Name** | **Sequence 5’ -3’** |
| prME | ZIKV 1087 FWD (10 µM) | CCGCTGCCCAACACAAG |
| ZIKV 1163c REV (10 µM) | CCACTAACGTTCTTTTGCAGACAT |
| ZIKV 1108 FAM/no quencher | AGCCTACCTTGACAAGCAGTCAGACACTCAA |
| Capsid | ZIKV 162 FWD (10 µM) | GAGATTCACGGCAATCAAG |
| ZIKV 287 REV (10 µM) | CTTCTTCTCCTTCCTAGCAT |
| ZIKV 2042 FAM/BHQ | /56-FAM/ATTCTCAGCATGGCAGCCAGATCT/3BHQ\_1/ |
| NS2b | ZIKV 4218 FWD (10 µM) | GGTCGGTCTGCTAATTGT |
| ZIKV 4304 REV (10 µM) | CAGTGACTTCCGCATCTT |
| ZIKV 4240 FAM | /56-FAM/ACGTGGTCTCAGGAAAGAGTGTGGA/3BHQ\_1/ |