**Print Only Page 2-3 for Customer**

**Concentration:** rEVAlution qPCR MasterMix: 2X, ROX Reference Dye: 25μM

**Storage and Handling:**

Upon arrival store at -20°C for provided expiration date, Room Temperature for 30 Days, 4°C for up to 60 days. Minimize Freeze thaw of master mix to avoid loss of performance.

**Ordering Information:**

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| **Item Number** | **Number of Tubes and Volume** | **Total number of reactions which can be obtained when using the following reaction sizes** |
| **20μL Reaction** | **10μL Reaction** |
| RQ -500 | rEVAlution 2X qPCR MasterMix: 5 x 1mL, 25μM ROX Reference Dye: 1x150uL | 500 | 1000 |
| RQ -1000 | rEVAlution 2X qPCR MasterMix: 10x1mL, 25μM ROX Reference Dye: 2x150uL | 1000 | 2000 |
| RQ -2500 | rEVAlution 2X qPCR MasterMix: 25x1mL, 25μM ROX Reference Dye: 5x150uL | 2500 | 5000 |

**Product Description:**

Empirical’s rEVAlution is 2X concentrated and ready to use hot-start qPCR MasterMix with EvaGreen® dye, FlashTaq DNA polymerase, dCTP, dGTP, dATP, dTTP, and MgCl2. EvaGreen® is a dsDNA-binding dye. It is cell membrane impermeable, non-cytotoxic, non-mutagenic, and safe for aquatic life. EvaGreen­® possesses similar absorption and emission spectra as SYBR® Green I.

FlashTaq is an exceptional chemically modified hot-start Taq DNA polymerase with an activation time of 2 minutes at 95°C. Taq DNA Polymerase gene is isolated from *Thermus aquaticus* YT1 and expressed in *E. coli*.

A 25µM ROX reference dye is supplied separately for use with some real time machines.

**Protocol:** Minimize Freeze thaw of master mix to avoid loss of performance. The following reaction set up and general cycling conditions are recommended but can vary depending on the template and primers being used.

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| **Table 1: Recommended Protocol for 20uL Reaction** |
| Component | Volume | Final Concentration |
| rEVAlution 2X qPCR MasterMix | 10 µl | 1X |
| ROX reference dye, 25μM | X µl | Table 2 |
| Primer, 10µM | X µl | 0.1-1.0µM |
| DNA Template | X µl | > 0.1ng |
| Nuclease Free Water to volume | X µl | N.A. |
|  |
| **Table 2: ROX concentration recommendation for different instrument** |
| Type | Company | Instrument | Final Conc. |
| No ROX | Roche | LightCycler 480, LightCycler 2.0 | None |
| BioRad | iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, Chromo4, MJ Opticon, Option2, MiniOpticon |
| Qiagen | Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000 |
| Illumina | Eco RealTime PCR System |
| Eppendorf | Mastercycler realplex |
| Cepheid | SmartCyler |
| Low ROX | ABI | 7500, 7500 Fast | 30nM |
| Stratagene | MX4000P, MX3000P, MX3005P |
| High ROX | ABI | 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus, Viia7 | 300nM |

**Recommended Thermal cycling conditions:** The following general cycling conditions are recommended but can vary depending on the template and primers being used.

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| **Table 3: Cycling Conditions** |
| Cycling Step | Temperature | Holding Time | Cycles |
| Enzyme activation | 95°C | 2min | 1 |
| Denaturation | 95°C | 5sec |  40 |
| Annealing# | 50-65°C | 5sec – 20sec |
| Extension | 72°C | 25sec |
| #Annealing will depend on primer length and composition. Generally, begin 5°C below primer Tm. |

**Notice to Purchaser:**

SYBR® is a registered trademark of Invitrogen, Inc. EvaGreen® is a registered trademark of Biotium, Inc. Empirical Bioscience is licensed by Biotium, Inc. to sell reagents including EvaGreen® dye, for research purposes only. Use of this product is covered by the following US patents and foreign equivalents: US7,803,943 B2, US7,776, 567, B2, including any patent application from which any of the listed patents directly or indirectly matured, any divisional, continuation and continuation-in-part application of any of the foregoing patent applications.