

Product Information	
PerfeCTa® Multiplex qPCR ToughMix, ROX™	
Part Number	95148-250
Unit Size	250 x 20-µL Rxn.
Storage Temperature	-25°C to -15°C
Lot Number	66155543
Reference Number	022920
Expiration Date	02/28/2022

**Product Description:**

PerfeCTa MultiPlex qPCR ToughMix is a 5X concentrated, ready-to-use reaction cocktail for real-time quantitative PCR (qPCR) with ToughMix reagent technology that neutralizes a broad spectrum of PCR inhibitors that compromise assay performance with crude extracts, clinical specimens, plant, soil environmental or complex food matrix. The only necessary user-supplied materials are probe assays and DNA samples. Extra-concentrated reagent provide more flexibility with dilute DNA samples sensitivity. PerfeCta MultiPlex qPCR ToughMix has been rigorously optimized to deliver maximum assay precision, sensitivity and PCR efficiency in miniaturized reaction volumes with either conventional or accelerated thermal cycling conditions.

Suppression of low copy amplicons by high copy reference targets during multiplex co-amplification skews the apparent representation and quantification of low copy target sequences. PerfeCTa MultiPlex qPCR ToughMix transcends these limitations by enabling 6 orders of magnitude in sensitive, linear assay performance with concurrent amplification of four abundant targets at 106 each. PerfeCTa MultiPlex qPCR ToughMix results in multiplexed qPCR with dynamic range and sensitivity that are comparable to single-plex qPCR probe assays without the need to rigorously titrate primer concentration.

A key component of PerfeCTa® MultiPlex qPCR ToughMix® is an ultra pure, highly processive thermostable DNA polymerase that is combined with high avidity monoclonal antibodies. This proprietary polymerase mix is highly resistant to PCR inhibitors and provides an extremely stringent automatic hot-start allowing reaction assembly, and temporary storage, at room temperature prior to PCR amplification.

Product Specifications				
95148				
Assay	Multiplex qPCR Functional Assay	High Biased, Multiplex qPCR	DNase	RNase
Result	Pass	Pass	Pass	Pass

**Component Part Numbers:**

84265 Multiplex qPCR ToughMix ROX, 1.25mL

**Quality Control Analysis and Specifications:**

**Multiplex qPCR:** 4-color multiplex qPCR is performed in triplicate reactions on 10-fold serial dilutions of an equal copy mixture of four plasmid DNAs (1 x10<sup>7</sup> copies to 10 copies). Cq standard curve analysis for each targeted sequence must have a coefficient of determination (r<sup>2</sup>) ≥0.990 with a slope between -3.25 and -3.65. Control reactions lacking template DNA (NTC) must remain below fluorescence threshold through 45 PCR cycles.

**High Biased, Multiplex qPCR:** 4-color multiplex qPCR of a 10-fold serial dilution of a FAM probe specific plasmid DNA (1 x10<sup>7</sup> copies to 10 copies) in a fixed background of 3 different plasmid targets at 1x10<sup>8</sup> copies (each) must have an LOD of 10 copies. Cq standard curve analysis must have a coefficient of determination (r<sup>2</sup>) ≥0.990 with a slope between -3.25 and -3.70.

**DNase:** DNase activity must be below the detectable limits of 100 pg DNase I equivalent as assayed using a fluorogenic substrate following a 1 hour incubation at 37°C with each kit component: 2X qScript RT, 1X qScript Reaction Mix, or nuclease-free water and control buffer.

**RNase:** RNase activity must be below the detectable limits of 1 pg RNase A equivalent as assayed using a fluorogenic substrate following a 1 hour incubation at 37°C with each kit component: 2X qScript RT mix, 1X qScript Reaction Mix, or nuclease-free water and control buffer.

**Limitations of Use**

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