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Technical Note

Detection of *Salmonella* Using Spex 2-Color *Salmonella* Quantitative PCR (qPCR) Kit

There are over 300 million cases of foodborne illnesses in the world each year resulting in almost half a million deaths. *Salmonella* causes almost 80 million of those illnesses, with several hundred deaths each year. *Salmonella* are rod-shaped, gram-negative bacteria from the Enterobacteriaceae family. There are two species of *Salmonella* (*S. enterica* and *S. bongori*) with six subspecies and over 2,600 serotypes or strains; some (mostly typhoidal strains) can cause severe illness or death. The 2-Color *Salmonella* qPCR kit is designed to detect the presence of *Salmonella* species DNA in tissues of agricultural samples including cannabis products.

Materials

Samples must be homogenized, and the nucleic acids extracted prior to use with the *Salmonella* test kit. See Spex application notes for assistance on proper sample homogenization and the use of the Spex DNAmax Extraction Kit.

The Spex 2-Color *Salmonella* Quantitative PCR (qPCR) Kit includes the following components:

- **Primers and Probes**
  The primers and probes included in the kit are designed to target the *invA* gene which encodes essential proteins for pathogenicity associated with *Salmonella* infection. The use of *invA* gene for *Salmonella* detection is in line with FDA requirements. Importantly, the *invA* gene has been extensively evaluated for inclusivity with DNA from over 600 strains of *Salmonella* species and over 200 non-*Salmonella* species.

- **Master Mix** (with recombinant Hot-Start Taq DNA polymerase)
  The 2-Color *Salmonella* kit comes equipped with a master mix that includes a recombinant Taq DNA polymerase, an aptamer-based hot start technology to inhibit non-specific amplification, and a cocktail of PCR enhancers that promote a specific and efficient amplification of *Salmonella* species DNA. The master mix incorporates an inert blue dye which, in combination with a yellow dye that is added to the DNA sample, permits visual confirmation that all components are mixed as well as easy loading of the master mix into wells of a qPCR plate.

- ***Salmonella* Positive Control**
  The *Salmonella* positive control contains a small synthetic PCR fragment from *Salmonella* DNA cloned into a TA vector. The plasmid is then linearized for use as a positive control in qPCR.

- **Internal Control Template DNA**
  The internal control contains a synthetic DNA fragment optimized for use in qPCR. This DNA fragment is also cloned into TA vectors and linearized.

- **ROX**
  This is a reference dye used for well-to-well normalization during qPCR.

- **Yellow Dye (20x)**
  This unique yellow dye is added to the DNA sample and changes from yellow to green to indicate that all components necessary for qPCR are present.

- **Water** (DNase-, RNase-, Protease-free)

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Method Protocol

Please review the detailed protocol that comes with the kit. The kit should be removed from storage in the freezer and given time to fully thaw on ice. Before first use, ROX is added to the **2X *Samonella* Detection Master Mix** to ensure qPCR machine compatibility. For a high ROX instrument, add the entire contents of the vial. For a low ROX instrument, use 1/10th the contents. Mix all tubes thoroughly to ensure homogeneity of all components.

Afterwards, 10 µL of the **2X *Salmonella* Detection Master Mix** is added to the designated wells of a qPCR plate. The following components are mixed in a separate 1.5 mL tube:

Component	Volume (10 µL)
20X Yellow Dye	1 µL
DNA template or *Salmonella* Positive Control Template*	1–6 µL
Internal Control Template	2 µL
Water	up to 10 µL

** For the positive control, replace the DNA template with 2 µL of the Salmonella positive control template. For negative control, do not add any DNA template.*

After addition of the components, centrifuge briefly (5–10 seconds) and pipette into wells containing the **2X *Salmonella* Detection Master Mix** and prepare the qPCR.

qPCR is performed using the following conditions:

Step	Temperature (°C)	Time	Number of Cycles
Denature	95	1 minute	1
qPCR Detection	95	10 seconds	35
	60	30 seconds	

Results and Discussion

Salmonella DNA is detected via the FAM channel, whereas the internal PCR control is detected via the HEX or VIC channel. The limit of detection was determined to be less than 100 copies of *Salmonella* species DNA. Importantly, the primers and probes in the master mix generate a linear increase in cycle-threshold values throughout the entire dilution range (Figure 1). Analysis of the cycle-threshold values versus negative Log_{10} of *Salmonella* DNA copy number was also performed. An R-squared value of 0.99 was observed over the entire dynamic range, indicating that primers and probes are optimized.

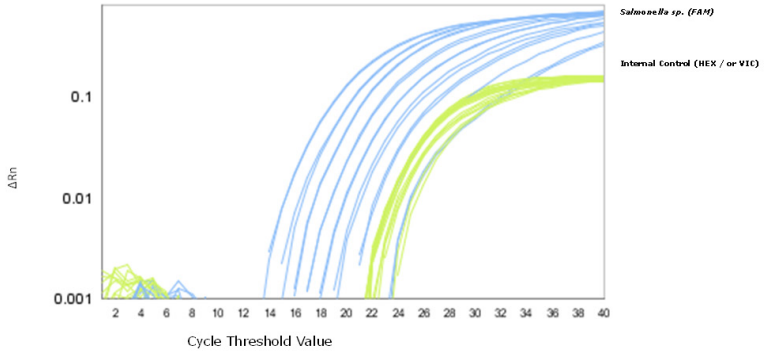

Figure 1. Serial dilution of *S. enterica* DNA to determine limit of detection.

Salmonella enterica genomic DNA was two-fold serially diluted and 2 µL of the diluted DNA was used for qPCR using the 2-color *Salmonella* detection master mix (FAM channel). Internal control DNA was detected via the HEX or VIC channel.

Results from the qPCR should be available within 45–60 minutes depending on the ramp rates of the qPCR machine. The figure below demonstrates results that can be expected when using the 2-color *Salmonella* qPCR kit.

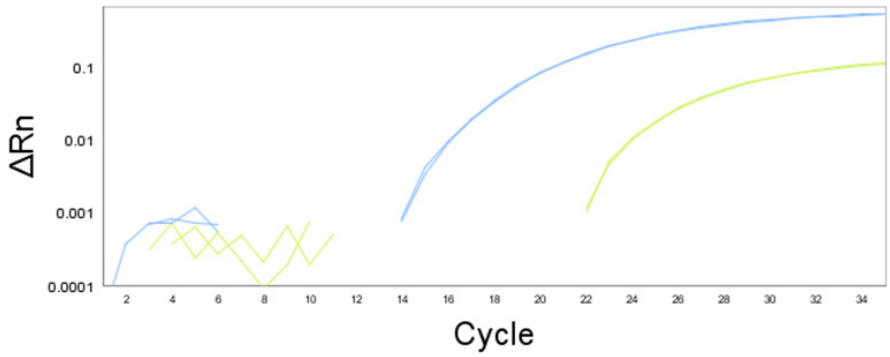

Figure 2. Expected results when using the 2-Color *Salmonella* qPCR Kit.

The positive and internal control template activates the FAM and HEX channels, respectively. For machines that lack a HEX channel, the VIC channel can be interchangeably used.

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