XP-Design Assay *Salmonella* Kentucky - Technical Report

June 2025

This Technical Report, specific to the XP-Design Assay *Salmonella* Kentucky, is intended to be used as a complement to the XP-Design Assay User Guide (document #10000241371).

Assay Information

Assay Name	XP-Design Assay *Salmonella* Kentucky
Assay Type	TaqMan
Catalog Number	qFSD51560321
Assay Description	The XP-Design Assay *Salmonella* Kentucky is a ready-to-use primer and probe solution for the qualitative detection of a specific DNA sequence unique to *Salmonella* Kentucky, in isolated colonies or in food and environmental samples

Scope of Use

Products used to generate technical data:

Real-Time PCR Primer-Probe Mix	XP-Design Assay *Salmonella* Kentucky
Real-Time PCR Supermix	iQ Multiplex Powermix (catalog #17007081)
Real-Time PCR Instruments	CFX96 Touch Deep Well Real-Time PCR System CFX Opus Deepwell Real-Time PCR System
Experimental Samples	• Isolated *Salmonella* colonies • Food and environmental samples: chicken carcass rinse, turkey sponge swabs, poultry boot swabs, beefsteak

Protocol used for the technical study on isolated colonies:
(refer to the XP-Design Assay User Guide for detailed information on the protocol)

Bacterial Culture	*Salmonella* colonies were grown on nonselective agar plates
DNA Extraction	DNA was extracted from the isolated *Salmonella* colonies as described in the XP-Design Assay User Guide, section B: “Confirmation of single colonies using XP-Design Assays”
Fluorophore	FAM
Thermal Cycler Protocol	1 95.0 C for 3:00 2 95.0 C for 0:15 3 58.0 C for 0:20 + Plate Read 4 GOTO 2 , 29 more times END

Protocol used for technical study on food and environmental samples:
(refer to the XP-Design Assay User Guide for detailed information on the protocol)

Enrichment Scheme	Enrichment of the food and environmental samples was carried out as described in the iQ-Check *Salmonella* spp. II User Guide (document #10000131519, section 7A)
DNA Extraction	DNA was extracted from the enrichments following the Easy protocol of the iQ-Check *Salmonella* spp. II method (refer to document #10000131519, section 7C)
Fluorophore	FAM
Thermal Cycler Protocol	1 95.0 C for 3:00 2 95.0 C for 0:15 3 58.0 C for 1:00 + Plate Read 4 GOTO 2 , 49 more times END

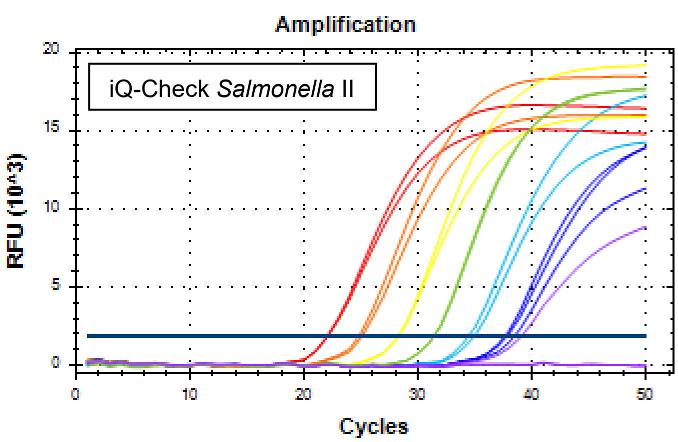
Experimental Testing of the XP-Design Assay *Salmonella* Kentucky

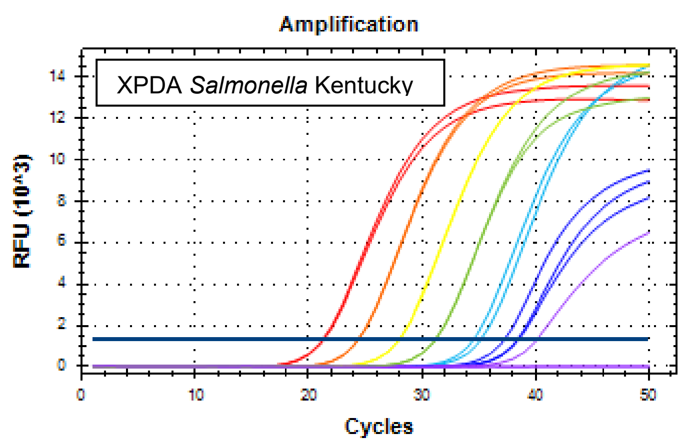
Specificity	The specificity of the *Salmonella* Kentucky primers and probe was assessed *in silico* on a library of 6,267 genomes, representing more than 200 serotypes. The PCR system was then tested *in vitro*, with high-throughput microfluidic qPCR, on a collection of 900 strains, representing 72 serotypes or lineages of *Salmonella enterica* subsp. *enterica*, the 5 other *Salmonella enterica* subspecies and *Salmonella bongori*, and 8 non-*Salmonella* bacterial species. Finally, the specificity of the XP-Design Assay *Salmonella* Kentucky was verified on a collection of 61 strains, representing 47 serotypes or lineages of *Salmonella enterica* subsp. *enterica*.

Limit of Detection	Similar to iQ-Check *Salmonella* II
Efficiency	97% Assay efficiency was determined using a six-point standard curve generated from 10-fold serial dilutions of a target DNA. An efficiency of 100% represents a perfect doubling of template at every cycle. Acceptance criteria was 90-110%, the typical range of good assay efficiency
R^2	0.9971 The R^2 represents the linearity of the standard curve and how well the standard curve data points fit the linear regression line. Acceptable values were > 0.99
Robustness to inhibition	Artificially spiked enrichment extracts were used to verify that target detection was not impeded by matrix inhibition. Matrices used for this purpose: chicken carcass rinse, turkey sponge swabs, poultry boot swabs, beefsteak.

Experimental results obtained with the XP-Design Assay *Salmonella* Kentucky:

Amplification curves of a DNA extract from *Salmonella* Kentucky CIP 105623, serially diluted in H_2O, tested in parallel with iQ-Check *Salmonella* II and with XP-Design Assay *Salmonella* Kentucky





The following table shows the Cq values obtained for each concentration level of target (N/A: no amplification detected):

		Cq values obtained					
		iQ-Check *Salmonella* II			**XPDA *Salmonella* Kentucky**		
	Dilution factor	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 1**	**Replicate 2**	**Replicate 3**
	10	22.07	22.01	Not tested	21.30	21.21	Not tested
	100	25.05	24.76	Not tested	24.35	24.36	Not tested
	1E+03	28.22	28.25	Not tested	28.02	27.98	Not tested
	1E+04	31.32	31.38	Not tested	31.19	31.14	Not tested
	1E+05	34.93	34.38	Not tested	34.50	35.18	Not tested
	1E+06	38.40	37.73	37.56	38.46	38.36	37.24
	1E+07	39.03	N/A	N/A	N/A	N/A	40.11

XP-Design Assays are not experimentally wet-lab validated.
They should be validated prior to use by the end-user.

Bio-Rad Laboratories, Inc., Food Science Division, www.bio-rad.com/FoodScience