XP-Design Assay *E. coli* O80 O-target
Technical Report

March 2025

This Technical Report, specific to the XP-Design Assay *E. coli* O80 O-target, is intended to be used as a complement to the XP-Design Assay User Guide (document #10000241371).

Assay Information

Assay Name	XP-Design Assay *E. coli* O80 O-target
Assay Type	TaqMan
Catalog Number	qFSD80365367
Assay Description	The XP-Design Assay *E. coli* O80 O-target is a ready-to-use primer and probe solution for the qualitative detection of specific DNA sequences unique to *Escherichia coli* serogroup O80, 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 *E. coli* O80 O-target
Real-Time PCR Supermix	iQ Multiplex Powermix (catalog #17007081)
Real-Time PCR Instrument	CFX Opus 96 Real-Time PCR System
Experimental Samples	• *E. coli* pure cultures • Food samples from the following categories: flour and raw dough, spinach, raw beef and multicomponent foods and meal components

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

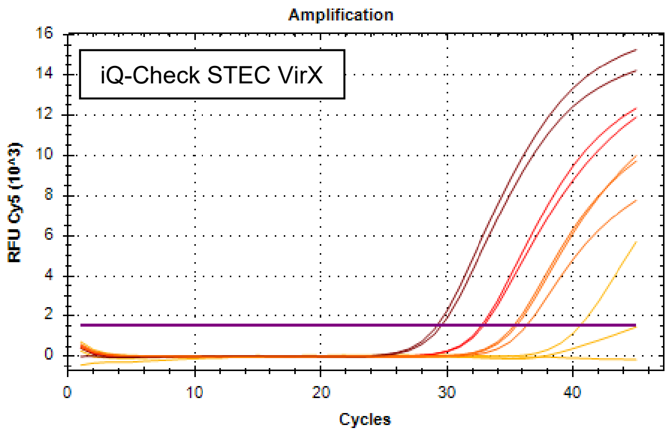
Bacterial Culture	*Escherichia coli* strains were grown in BPW medium for 18-24h at 37°C
Enrichment Scheme	Enrichment of the food samples was carried out as described in the iQ-Check STEC VirX User Guide (document #10000131516, section 7A)
DNA Extraction	DNA was extracted from the pure cultures or from the enrichments following the Easy protocol of the iQ-Check STEC VirX method (refer to document #10000131516, section 7C)
Fluorophore	FAM
Thermal Cycler Protocol	The iQ-Check STEC SerO II Fast Application Protocol File (APF) * and the thermoprofile below on the User Defined Setup are both verified with the XP-Design Assay *E. coli* O80 O-target: 1 95.0 C for 10:00 2 95.0 C for 0:02 3 58.0 C for 0:15 + Plate Read 4 72.0 C for 0:08 5 GOTO 2 , 44 more times END *If using the SerO II Fast APF, the final PCR results will all indicate “invalid” due to the lack of an internal control in the XP-Design Assays. The end user will have to manually interpret the results using the Cq value in the FAM channel.

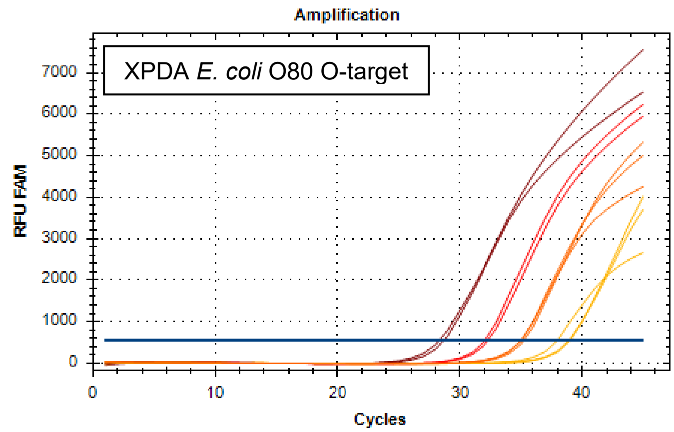
Experimental Testing of the XP-Design Assay *E. coli* O80 O-target

Specificity	The specificity of the XP-Design Assay *E. coli* O80 O-target was verified on 9 *E. coli* O80 strains and 10 non-O80 STEC strains
Limit of Detection	Similar to iQ-Check STEC VirX
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: spinach and beef steak.

Experimental results obtained with the XP-Design Assay *E. coli* O80 O-target:

Amplification curves of a DNA extract from an *eae*-positive *E. coli* O80 strain, serially diluted in sterile water, tested in parallel with iQ-Check STEC VirX and with XP-Design Assay *E. coli* O80 O-target





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

		Cq values obtained					
	Dilution factor	iQ-Check STEC VirX (Cy5 channel: *eae* target)			XPDA *E. coli* O80 O-target (FAM channel)		
		Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3
	1E+04	29.56	29.23	Not tested	28.62	28.26	Not tested
	1E+05	32.96	32.76	Not tested	32.25	32.02	Not tested
	1E+06	35.33	36.33	35.53	35.19	34.99	35.00
	1E+07	40.57	N/A	N/A	37.94	38.86	38.98

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

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Website *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *00 800 00 24 67 23* ***Belgium*** *00 800 00 24 67 23* ***Brazil*** *4003 0399* ***Canada*** *1 800 361 1808* ***China*** *86 21 6169 8500* ***Czech Republic*** *00 800 00 24 67 23* ***Denmark*** *00 800 00 24 67 23* ***Finland*** *00 800 00 24 67 23* ***France*** *00 800 00 24 67 23* ***Germany*** *00 800 00 24 67 23* ***Hong Kong*** *852 2789 3300* ***Hungary*** *00 800 00 24 67 23* ***India*** *91 124 4029300* ***Israel*** *0 3 9636050* ***Italy*** *00 800 00 24 67 23* ***Japan*** *81 3 6361 7000* ***Korea*** *82 080 007 7373* ***Luxembourg*** *00 800 00 24 67 23* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *00 800 00 24 67 23* ***New Zealand*** *64 9 415 2280* ***Norway*** *00 800 00 24 67 23* ***Poland*** *00 800 00 24 67 23* ***Portugal*** *00 800 00 24 67 23* ***Russian Federation*** *00 800 00 24 67 23* ***Singapore*** *65 6415 3188* ***South Africa*** *00 800 00 24 67 23* ***Spain*** *00 800 00 24 67 23* ***Sweden*** *00 800 00 24 67 23* ***Switzerland*** *00 800 00 24 67 23* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *36 1 459 6150* ***United Kingdom*** *00 800 00 24 67 23*