

Cole-Parmer® qPCR test

Universal Fish Fish

Speciation Kit

100 tests

For general laboratory and research use only

Cole-Parmer®

Kit Contents

- **Universal Fish specific primer/probe mix (BROWN)**
FAM labelled
- **Universal Fish positive control template (RED)**
- **Internal extraction control primer/probe mix (BROWN)**
VIC labelled as standard
- **Internal extraction control DNA (BLUE)**
- **RNAse/DNAse free water (WHITE)**

Reagents and equipment to be supplied by the user

Real-Time PCR Instrument

DNA extraction kit

This kit is designed to work well with all processes that yield high quality DNA with minimal PCR inhibitors.

Lyophilised 2x qPCR Mastermix

This kit is designed to be compatible with all commercially available Mastermixes that run with standard cycling conditions.

Pipettors and Tips

Vortex and centrifuge

Thin walled 1.5 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20°C on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Cole-Parmer does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and DNA integrity (An internal PCR control is supplied to test for non specific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative-control, replace the template DNA sample with RNase/DNase free water.

Dynamic range of test

Under optimal PCR conditions Cole-Parmer Universal Fish detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and Disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Cole-Parmer detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applied Biosystems Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S.

Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc. and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks

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The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. BI, ABI PRISM®, GeneAmp® and MicroAmp® are registered trademarks of the Applied Biosystems (Applied Biosystems Corporation). BIOMEK® is a registered trademark of Beckman Instruments, Inc.; iCycler™ is a registered trademark of Bio-Rad Laboratories, Rotor-Gene is a trademark of Corbett Research. LightCycler™ is a registered trademark of the Idaho Technology Inc. GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc., The purchase of the Cole-Parmer reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-La Roche Inc.

Reconstitution Protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

- 1. Pulse-spin each tube in a centrifuge before opening.**
This will ensure lyophilised primer and probe mix is in the base of the tube and is not spilt upon opening the tube.
- 2. Reconstitute the kit components in the RNase/DNase free water supplied according to the table below:**
To ensure complete resuspension, vortex each tube thoroughly.

Component	Volume
Pre-PCR pack	
Universal Fish primer/probe mix (BROWN)	110 μ l
Internal extraction control primer/probe mix (BROWN)	165 μ l
Pre-PCR heat-sealed foil	
Internal extraction control DNA (BLUE)	500 μ l
Post-PCR heat-sealed foil	
Universal Fish Positive Control Template (RED) *	500 μ l

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

DNA extraction

The internal extraction control DNA can be added either to the DNA lysis/extraction buffer or to the DNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

- 1. Add 4 μ l of the Internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer per sample.**
- 2. Complete DNA extraction according to the manufacturers protocols.**

Real-time PCR detection protocol

1. **For each DNA sample prepare a reaction mix according to the table below:**
Include sufficient reactions for positive and negative controls.

Component	Volume
Lyophilised 2x qPCR Mastermix	10 μ l
Universal Fish primer/probe mix (BROWN)	1 μ l
Internal extraction control primer/probe mix (BROWN)	1 μ l
RNAse/DNAse free water (WHITE)	3 μ l
Final Volume	15 μl

2. **Pipette 15 μ l of each mix into individual wells according to your real-time PCR experimental plate set up.**

3. **Pipette 5 μ l of DNA template into each well, according to your experimental plate set up.**

To obtain a strong signal, the ideal concentration of DNA is 1-3ng/ μ l. The concentration should not exceed 5ng/ μ l. Substitute sample DNA for RNAse/DNAse free water as a negative control. Substitute sample DNA for positive control template as a positive control.

Amplification Protocol

Amplification conditions using lyophilised 2x qPCR Mastermix.

	Step	Time	Temp
	UNG treatment (if required) **	15 mins	37 °C
	Enzyme activation	2 mins	95 °C
Cycling x50	Denaturation	10 secs	95 °C
	DATA COLLECTION *	60 secs	60 °C

* Fluorogenic data should be collected during this step through the FAM and VIC channels

** Required if your Mastermix includes UNG to prevent PCR carryover contamination

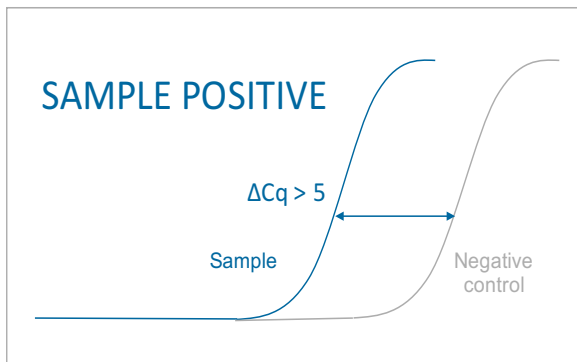
Interpretation of Results

For interpretation when speciation kit used independently

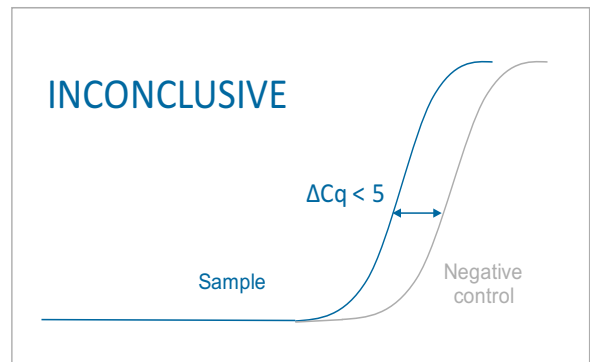
Target (FAM)	Internal control (VIC)	Positive control	Negative control	Interpretation
≤ 35	+ / -	+	-	POSITIVE RESULT
> 35 or -	+	+	-	NEGATIVE RESULT
+ / -	+ / -	+	≤ 35	EXPERIMENT FAILED due to test contamination
+ / -	+ / -	+	> 35	*
> 35 or -	-	+	-	SAMPLE PREPARATION FAILED
+ / -	+ / -	-	+ / -	EXPERIMENT FAILED

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

*Where the test sample is positive and the negative control is positive with a Cq > 35 , the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Internal PCR control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of RNA added to the RT and PCR reaction and the individual machine settings. Cq values of 28 ± 3 are within the normal range. When amplifying a Fish sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

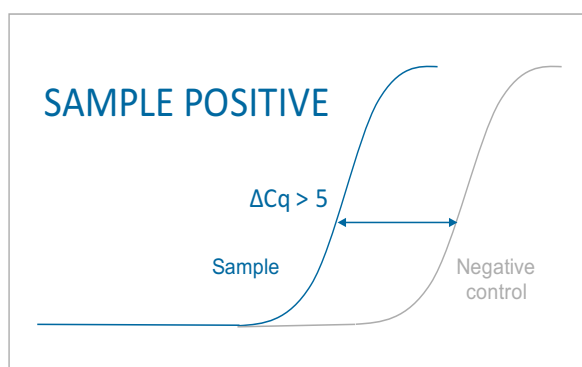
Interpretation of Results

For interpretation when speciation kit used in conjunction with the universal meat or fish kit

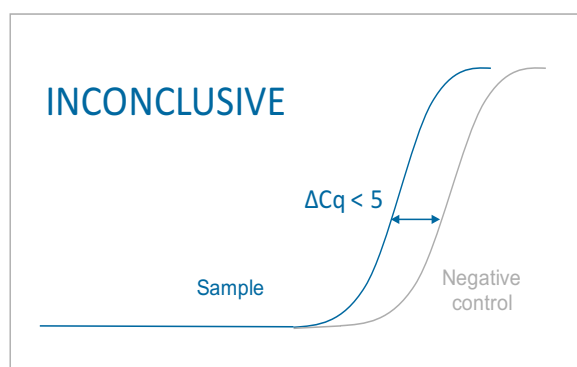
Target (FAM)	Internal control (VIC)	Universal signal (FAM)	Positive control (Target & Universal signal)	Negative control (Target only)	Interpretation
+	+/-	+	+	-	POSITIVE RESULT calculate species % and check test sensitivity
-	+	+	+	-	NEGATIVE RESULT
-	-	+	+	-	SAMPLE FAILED repeat test
+	+/-	-	+	-	SAMPLE FAILED repeat test
+/-	+/-	+/-	+	≤ 35	EXPERIMENT FAILED due to test contamination
+/-	+/-	+/-	+	> 35	*
-	+	-	+	-	NO ANIMAL DNA DETECTED
-	-	-	+	-	SAMPLE PREPARATION FAILED
+/-	+/-	+/-	-	+/-	EXPERIMENT FAILED

Positive control template (**RED**) is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised

*Where the test sample is positive and the negative control is positive with a Cq > 35, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 5 Cq earlier than the negative control then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 5 Cq earlier than the negative control then the positive sample result is invalidated and the result should be determined inconclusive due to test contamination. The test for this sample should be repeated.

Calculating species %

(n.b. a Microsoft Excel applet for automatic % calculation is available free of charge. Contact cptechsupport@coleparmer.com to request)

$$\text{Species \%} = (2^{-(\text{Cq Universal Fish [SAMPLE]} - \text{Cq Universal Fish [+ve control]})} - (2^{-(\text{Cq Universal meat [SAMPLE]} - \text{Cq Universal meat [+ve control]})})) \times 100$$

Worked example: test gives following Cq values:

Universal Fish test on sample: 23.5

Universal Fish test on positive control DNA: 24.1

Universal meat test on sample: 22.4

Universal meat test on positive control DNA: 22.2

Universal Fish% =

$$(2^{((23.5-24.1)-(22.4-22.2))}) \times 100 =$$

$$(2^{(-0.6 - 0.2)}) \times 100 =$$

$$(2^{-0.8}) \times 100 = \mathbf{57.4\%}$$

n.b. In rare circumstances, some samples may produce a speciation % greater than 100. This is usually due to the presence of PCR inhibition affecting the multiplex reaction and should be reported as 100%. If the reported speciation is greater than 400% then the level of PCR inhibition is likely too great for accurate speciation reporting. Samples such as these should be re-extracted with extra washes to remove PCR inhibitors.

Calculating test sensitivity

The sensitivity of a speciation test is dependant on the amount of DNA that has been successfully extracted from a given sample. The speciation kits have the unique ability to provide information on this sensitivity to empower the user to interpret their data with more precision.

Precise calculations on test sensitivity can be carried out using the Microsoft Excel applet for automatic % calculation that is available free of charge. (Contact cptechsupport@coleparmer.com to request). But as a rule of thumb the sensitivity of a given test can be estimated based upon the Cq value achieved from the Universal meat primer/probe.

Universal test Cq	Test sensitivity %
$Cq < 19.8$	0.01
$19.8 \leq Cq < 23.2$	0.1
$23.2 \leq Cq < 26.6$	1
$26.6 \leq Cq < 30.0$	10
$30.0 \leq Cq \leq 35.0$	Level of animal DNA is too low for accurate speciation testing
$Cq > 35.0$	Level of animal DNA is too low for analysis to proceed

If the calculated percentage of Universal Fish DNA is greater than the calculated test sensitivity then the quantitative result is accurate.

If the calculated percentage Universal Fish DNA is less than the calculated test sensitivity then the quantitative result is not accurate and a qualitative positive result equal to the reported % sensitivity should be reported

e.g. If your calculated percentage Universal Fish DNA is 1% but the calculated test sensitivity is only 10% then the quantitative result can not be assumed to be accurate. The qualitative result is still true however. i.e. the sample does contain Universal Fish DNA. But the percentage can only be assumed less than 10% rather than precisely 1%