Primerdesign™ Ltd

1% level DNA speciation controls

Instructions for the use of genesig®
1% level DNA speciation controls
in real-time PCR

GENESIG
Kits by Primerdesign

Contents

Introduction

The genesig 1% Level DNA controls are a high-quality source of DNA validated for use in qPCR experiments. The DNA has been extracted from animal tissue and analysed to confirm the absence of PCR inhibitors. Following this, the material is mixed with background DNA from an alternative animal source and subjected to a regime of quantitative tests before being packaged and supplied in a lyophilised format. Our 1% Level DNA Controls are a useful tool for confirming the accuracy of DNA speciation testing strategies.

Testing for meat adulteration poses many technical challenges regardless of the approach used. DNA testing via qPCR is the gold-standard for speciation, however one of its challenges is that meat (i.e. protein) content is not directly assayed; DNA levels are used as a surrogate marker instead. This can pose problems when attempting to validate the quantitative accuracy of DNA speciation tests. This is because 1% mixes of meat do not always translate to 1% at the DNA level. In these circumstances genesig 1% Level DNA Controls serve as an invaluable control material for qPCR speciation testing.

Kit contents

- **Species 1% Level DNA control (RED)**
- **Template preparation buffer (YELLOW)**
  For resuspension of positive control template
- **RNase/DNase free water (WHITE)**

Reagents and equipment to be supplied by user

- **Real-Time PCR instrument**
- **Master Mix or Master Mix components**
  This kit is designed to work well with all commercially available master mixes. However, we recommend the use of Primerdesign PrecisionPLUS or oasig 2X qPCR Master Mix.
- **genesig® speciation kit (standard, advanced or Easy)**
  If using the genesig q16 real time PCR machine you must use the genesig Easy kit.
- **Pipettors and tips**
- **Vortex and centrifuge**

Kit storage

The genesig 1% level DNA control kit should be stored at -20ºC on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20ºC for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. Under these conditions reagents are stable for six months from date of resuspension.
Primerdesign does not recommend using the kit after the expiry date stated on the pack

Primerdesign satisfaction guarantee

Primerdesign takes pride in the quality of all of our products. Should this product fail to perform satisfactorily when used according to the protocols in this manual, Primerdesign will replace the item free of charge.

Quality control

As part of our ISO9001 and ISO13485 quality assurance systems, all Primerdesign products are monitored to ensure the highest levels of performance and reliability.

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 Primerdesign genesig® 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 Applera 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.

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The purchase of the Primerdesign ™ reagents cannot be construed as an authorization or implicit license to practicePCR under any patents held by Hoffmann-LaRoche Inc

Resuspension protocol

1. **Resuspend 1% level DNA control in template preparation buffer provided according to the table below;**

   To ensure complete resuspension, vortex each tube thoroughly, allow to stand for 5 minutes and vortex again before use. The resuspended DNA can now be added directly to speciation qPCR tests.

Component	Volume
genesig 1% level DNA controls* (**RED**)	200µl

*This component contains high copy number template and is a significant contamination risk. It must be opened and handled in a separate laboratory environment.

qPCR detection protocol

The protocol below should be followed for customers using genesig standard or advanced speciation kit. If using a genesig EASY kit, please follow instructions on page 10.

1. **Setting up a genesig standard or advanced reaction:**
   Ensure that a negative and positive control is tested alongside the 1% Level DNA control. A suitable positive control is supplied with the genesig kit. For the negative control use the supplied water.

Component	1 Reaction
PrecisionPLUS or oasig Master Mix	10µl
genesig speciation primers/probe (**BROWN**)	1µl
RNase/DNase free water (**WHITE**)	4µl
Positive control or test sample or genesig 1% level DNA controls **(RED)**	5µl
Final volume	**20µl**

2. **Setting up a qPCR reaction using alternative supplier reagents:**
   Ensure that a negative and positive control is tested alongside the 1% Level DNA control. For the negative control use RNase/DNase free water.

Component	1 Reaction
Alternative master mix	X*µl
Alternative speciation kit	X*µl
RNase/DNase free water (**WHITE**)	X*µl
Positive control or test sample or genesig 1% level DNA controls **(RED)**	**5µl**
Final volume	**X*µl**

*Dependent on kit manufacturer's recommendation

qPCR amplification protocol

Amplification conditions for a genesig standard or advanced kit used with PrecisionPLUS or oasig 2X qPCR Master Mix

	Step	Time	Temp
	Enzyme activation	2min	95ºC
X 50 cycles	Denaturation	10s	95ºC
	DATA COLLECTION*	60s	60ºC

* Fluorogenic data should be collect during this step through the FAM channel.

Data interpretation for genesig®

When used according to the above protocols (assuming a 100% PCR efficiency), the 1% Level DNA speciation controls are expected to produce a speciation value of approximately 1% (an acceptable range for the reported speciation is 0.25 – 4%).

Please note, the expected speciation value can vary depending on the percentage speciation potency recorded on the certificate of analysis for your batch of 1% Level DNA Speciation Control. To calculate the exact percentage speciation range that should be applied to your batch, follow the steps below:

Upper limit = [Batch Percentage Potency] x 4
Lower limit = [Batch Percentage Potency] ÷ 4

Certificates of analysis and a speciation applet (for assistance in calculating speciation percentages) are available on request from support@primerdesign.co.uk**.**

If your speciation control does not produce the expected speciation value, this indicates that your speciation strategy is not performing optimally, please seek the appropriate technical support.

qPCR detection protocol

Setting up a genesig Easy reaction:

Ensure that a negative and positive control is tested alongside the 1% Level DNA control. A suitable positive control is supplied with the genesig EASY kit. For the negative control use the supplied water.

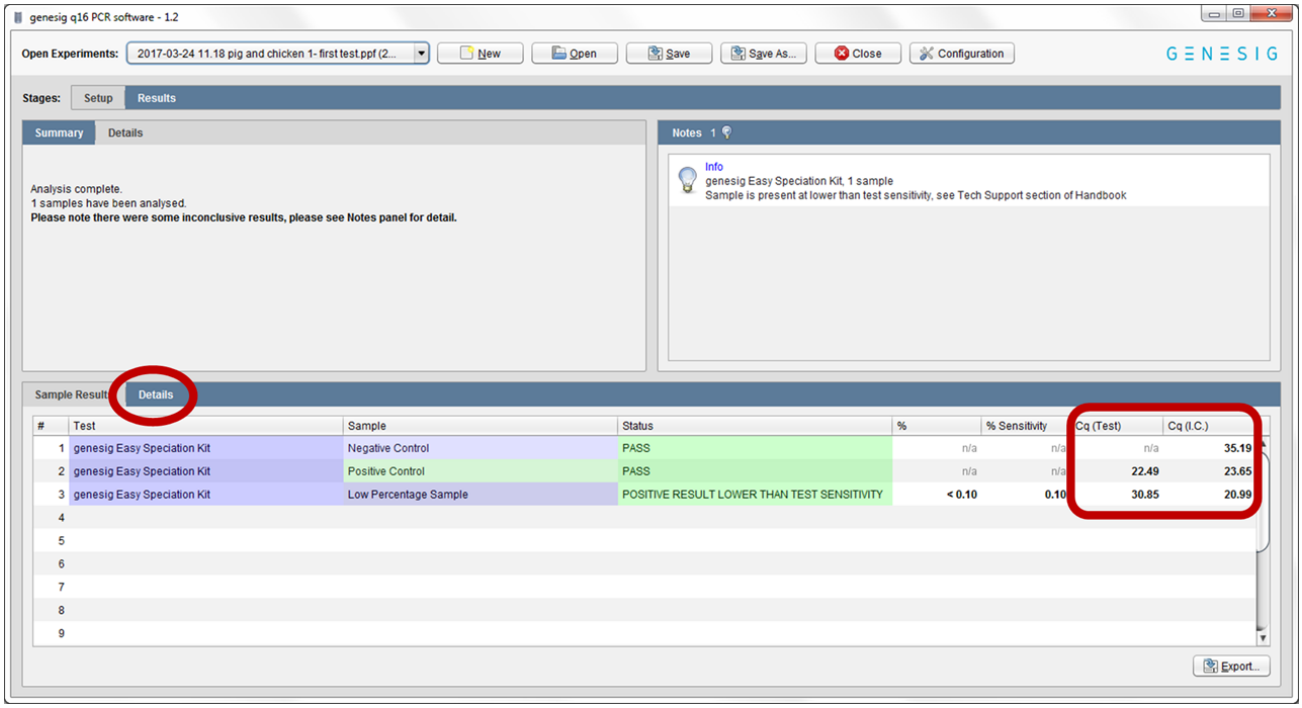
Component	1 reaction
genesig Easy speciation kit (**BROWN**)	10µl
Positive Control or Test Sample or genesig 1% Level DNA controls **(RED)**	10µl
Final volume	**20µl**

Data interpretation for genesig® Easy

When used according to the above protocol, the q16 will automatically calculate the percentage of the 1% Level DNA speciation control. The reported percentage should be approximately 1% (an acceptable range is 0.25 – 4%).

If the q16 reports “POSITIVE RESULT LOWER THAN TEST SENSITIVITY”, the exact speciation percentage will have to be calculated manually:

1. Click on the “Details” tab above the sample results table (indicated by the red circle in the figure below)
2. This will display the Cq values produced by the experiment on the right-hand side of the sample results table (indicated by the red square).
3. Input the Cq values into the speciation applet (available from support@primerdesign.co.uk.) to retrieve the exact speciation value.



Please note, the expected speciation value can vary depending on the percentage speciation potency recorded on the certificate of analysis for your batch of 1% Level DNA speciation control. To calculate the exact percentage speciation range that should be applied to your batch, follow the steps below:

Upper limit = [Batch Percentage Potency] x 4
Lower limit = [Batch Percentage Potency] ÷ 4

If your speciation control does not produce the expected speciation value, this indicates that your speciation strategy is not performing optimally, please seek the appropriate technical support. Contact support@primerdesign.co.uk