Primerdesign

Z-yDNA-hu-DD

Human Y chromosome detection kit with Double Dye probe

Kit version: 1

Target region: TSPY1 gene

150 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Product Description

This genesig® qPCR detection kit targets the TSPY1 gene in the human Y chromosome. The TSPY1 gene is not present in the X chromosome. The amplicon is present at multiple copies within the Y chromosome which increases the sensitivity for detecting very small amounts of genomic DNA and even degraded DNA.

Specificity

The kit is designed to detect the human Y chromosome and to have a broad detection profile. Specifically, the primers will detect over 95% of sequences available on the NCBI database at the time of last review.

This kit is predicted to cross-react with DNA from apes and monkeys.

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit, then please send an e-mail to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
1	**Human Y chromosome primer/probe mix (150 reactions)** FAM labelled		BROWN
1	**RNase/DNase free water** for resuspension of primer/probe mixes		WHITE

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Extraction kit

This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

Precision®PLUS, Precision®FAST or oasig®lyophilised 2X qPCR Master Mix

This kit is designed to work well with all commercially available master mixes. However, we recommend the use of Primerdesign oasig® lyophilised or PrecisionFAST® or PrecisionPLUS® 2X qPCR Master Mix.

Pipettors and filter tips

Vortex and centrifuge

1.5 ml microtubes

qPCR plates or reaction tubes

Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon 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.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA integrity.

Principles of the test

Real-time PCR

A target specific primer and probe mix is provided, and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labelled with a 5′-dye and a 3′-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

When resuspended, this kit provides primers that have been tested for priming specificity and amplification efficiency at optimal concentrations. qPCR is a very sensitive technology, and it is not recommended to use more or less than the specified amount of primer and probe in each reaction. However, final reaction volumes between 15µl and 50µl are often successful and may be tested at the user's discretion. Unfortunately, Primerdesign is not able to provide technical support for protocols other than those provided.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources of contamination should first be explored and removed.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting is 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/probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the kit components in the RNase/DNase free water supplied, according to the table below.**
   To ensure complete resuspension allow primer/probe mix to rehydrate for 10 minutes at room temperature. Vortex each tube thoroughly, followed by pipetting up and down 10 times. Failure to mix well can produce poor kit performance.

Component - resuspend in water	Volume
Pre-PCR pack	
Human Y chromosome primer/probe mix (**BROWN**)	165 µl

qPCR 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
oasig® lyophilised or Precision®FAST or PrecisionPLUS® 2X qPCR Master Mix	10 µl
Human Y chromosome primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15 µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Prepare sample DNA templates for each of your samples (suggested concentration 5ng/µl) in RNase/DNase free water.**
   If the concentration of DNA is not known, then dilute your DNA samples 1:20 (10µl of sample DNA and 190µl of water).

4. **Pipette 5µl of diluted template into each well, according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

qPCR Amplification Protocol

Please select the correct cycling protocol for the master mix that you are using.

Amplification conditions using oasig® lyophilised or PrecisionPLUS® 2X qPCR Master Mix.

	Step	Time	Temp
Cycling x40	Enzyme activation	2 min	95 °C
	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

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

Amplification conditions using PrecisionFAST® 2X qPCR Master Mix.

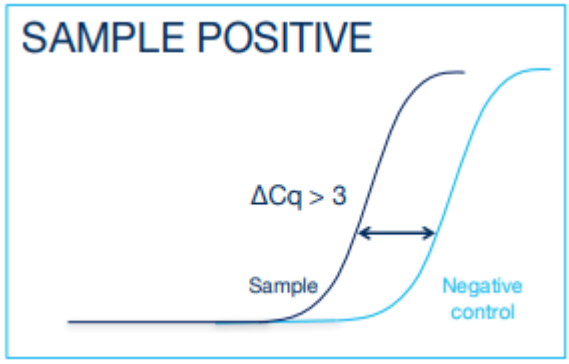
	Step	Time	Temp
Cycling x40	Enzyme activation	2 min	95 °C
	Denaturation	5 s	95 °C
	DATA COLLECTION *	20 s	60 °C

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

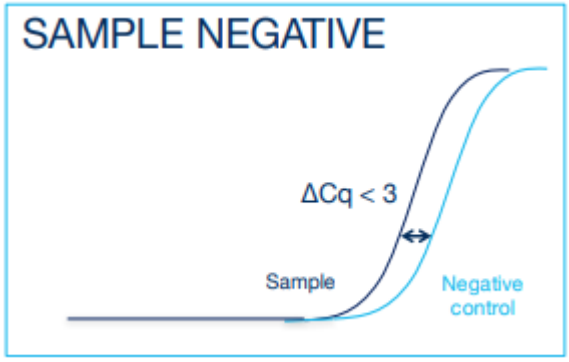
Interpretation of results

Target	Negative control	Interpretation
+	-	**POSITIVE RESULT**
-	-	**NEGATIVE RESULT**
+ / -	≤ 35	**EXPERIMENT FAILED** due to test contamination
+ / -	> 35	*

*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 > 3 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 < 3 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.

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 Primer Design Ltd 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 licence 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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