Primerdesign

Z-yDNA-hu-q-DD

Human Y chromosome quantification 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 kit provides reagents for the detection and quantification of 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 and quantify the human Y chromosome. 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	**Human gDNA positive control template** for standard curve		RED
1	**Internal extraction control primer/probe mix (150 reactions)** VIC labelled as standard		BROWN
1	**Internal extraction control DNA (150 reactions)**		BLUE
1	**RNase/DNase free water** for resuspension of primer/probe mixes		WHITE
2	**Template preparation buffer** for resuspension of internal control template, positive control template and standard curve preparation		YELLOW

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, 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.

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

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. An internal extraction control is provided to test for non-specific PCR inhibitors.

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 15 µl and 50 µl 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.

Positive control

For the purpose of DNA quantification, and as a positive control for the PCR set up, the kit contains a positive control genomic DNA. This can be used to generate a standard curve of Y chromosome concentration. Alternatively, the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false- positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

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. Human DNA is known to be highly prevalent within the air and environment generally and the negative control may therefore give a late positive signal due to environmental contamination. The interpretation of results section of this handbook gives guidance on how to interpret results where environmental contamination is evident.

Internal DNA extraction control

When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control DNA also indicates that PCR inhibitors are not present at a high concentration.

A separate primer and probe mix is supplied with this kit to detect the exogenous DNA using qPCR. The primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control DNA does not interfere with detection of the target DNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a Cq value of 28+/-3 depending on the level of sample dilution.

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
Internal extraction control primer/probe mix (**BROWN**)	165 µl

3. **Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
Positive Control Template (**RED**) *	100 µl
Pre-PCR heat-sealed foil	
Internal extraction control DNA (**BLUE**)	600 µl

* 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 µl of the Internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer.**

2. **Complete DNA extraction according to the manufacturer's protocols.**

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
Internal extraction control primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (WHITE)	3 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 (10 µl of sample DNA and 190 µl of water).

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

5. **Preparation of a 1:4 standard curve dilution series.**

   a. Pipette 90 µl of template preparation buffer (**YELLOW**) into 5 tubes and label them tube 2-6. The neat positive control tube (**RED**) is considered tube 1.

   b. Pipette 30 µl of positive control template (**RED**) into tube 2.

   c. Vortex thoroughly.

   d. Change pipette tip and pipette 30 µl from tube 2 into tube 3.

   e. Vortex thoroughly.

   Repeat steps d and e to complete the dilution series.

Standard Curve	DNA conc.	
Tube 1 Positive control (**RED**)	5000	pg/µl
Tube 2	1250	pg/µl
Tube 3	312	pg/µl
Tube 4	78	pg/µl
Tube 5	19	pg/µl
Tube 6	5	pg/µl

6. **Pipette 5 µl of the respective standard dilution into each well, according to your plate set up.**
   The final volume in each well is 20 µl.

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 and VIC channels.

Amplification conditions using PrecisionFAST® 2X qPCR Master Mix.

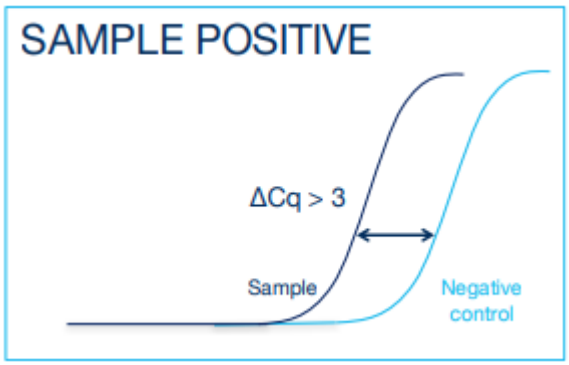
	Step	Time	Temp
Cycling x40	Enzyme activation	2 min	95 °C
	Denaturation	5 s	95 °C
	DATA COLLECTION *	20 s	60 °C

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

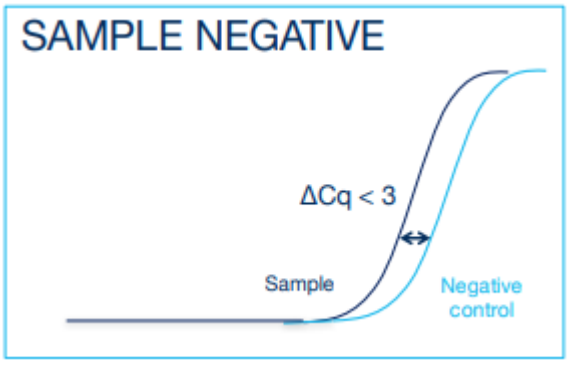
Interpretation of results

Target (FAM)	Internal control (VIC)	Negative control	Positive control	Interpretation
≤30	**+ / -**	**-**	**+**	**POSITIVE QUANTITATIVE RESULT** calculate DNA concentration
>30	**+**	**-**	**+**	**POSITIVE QUANTITATIVE RESULT** calculate DNA concentration
>30	**-**	**-**	**+**	**POSITIVE QUALITATIVE RESULT** Do not report DNA conc. as this may be due to poor sample extraction
-	**+**	**-**	**+**	**NEGATIVE RESULT**
+ / -	**+ / -**	**≤ 35**	**+**	**EXPERIMENT FAILED** due to test contamination
+ / -	**+ / -**	**> 35**	**+**	*****
-	**-**	**-**	**+**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**+ / -**	**-**	**EXPERIMENT FAILED**

*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 a negative call is the correct result.

Internal PCR control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a target 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.

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