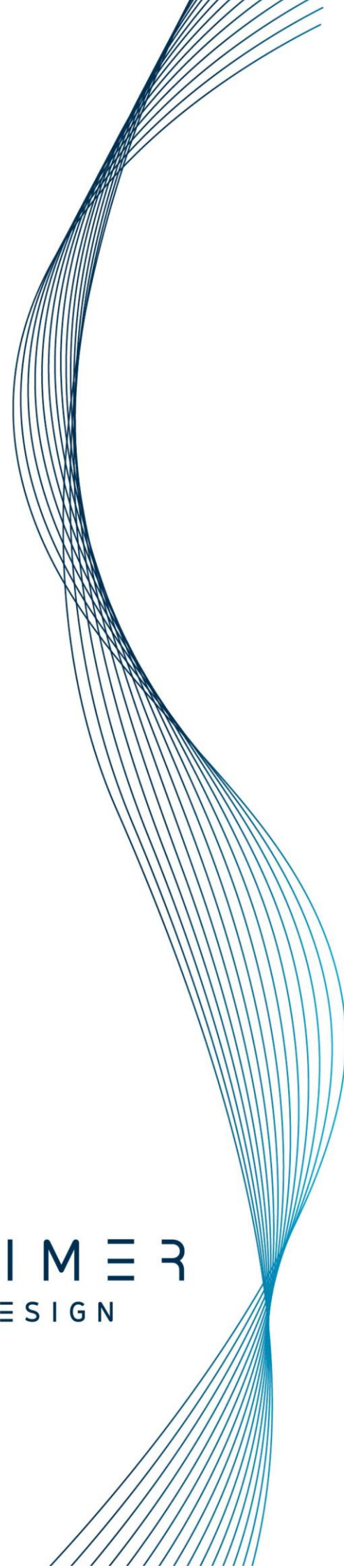


Human Y chromosome detection assay

Detection of the human Y chromosome by real-time PCR

PRIMER
DESIGN



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Kit contents

- Human Y chromosome primer/probe mix (150 reactions, **BROWN**)
- RNase/DNase free water (**WHITE**)

Reagents and equipment to be supplied by user

- Real-time PCR Instrument
- **Precision®PLUS, Precision®FAST or oasis™ 2X qPCR Master Mix**
This kit is designed to work well with all commercially available master mixes. However, we recommend the use of Primerdesign PrecisionPLUS, PrecisionFAST or oasis 2X qPCR Master Mix.
- Pipettors and Tips
- Vortex and centrifuge
- Thin walled 0.2 ml PCR reaction tubes

Kit storage and stability

This kit is stable at room temperature but should be stored at -20°C on arrival. Primerdesign does not recommend using the kit after the expiry date stated on the pack. Once the lyophilised components have been resuspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances

Suitable sample material

All kinds of sample material suited for qPCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration and DNA integrity. Always run at least one negative control with the samples. To prepare a negative control, replace the test sample with RNase/DNase free water.

Licensing agreement and limitations of use

PCR is covered by several patents owned by Hoffman-Roche Inc and Hoffman-LaRoche, Ltd. Purchase of Primerdesign kits does not include or provide licence with respect to any patents owned by Hoffman-La Roche or others.

Primerdesign satisfaction guarantee

Primerdesign takes pride in the quality of all 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

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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. The purchase of Biosearch Technologies products does not, either expressly or by implication, provide a license to use this or other patented technology. Licensing information can be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA 94404 or the Licensing Department at Roche Molecular Systems Inc., 1145 Atlantic Avenue, Alameda, CA 94501."

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

Trademarks

Primerdesign™ is a trademark of Primerdesign Ltd.

Precision® is a registered trademark of Primerdesign Ltd.

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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. ABI, 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.

Principles of the test

Introduction

This kit provides reagents for the detection of the human Y chromosome. The primers detect a region of the Y chromosome in the TSPY1 gene that 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.

Real-time PCR

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 μ l and 50 μ l 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 confirm absence of contamination, 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. 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.

Bench-side protocol

To minimise 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 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. Resuspend the kit components in RNase/DNase free water supplied, according to the table below:

To ensure complete resuspension, vortex each tube thoroughly.

Component	Volume
Y chromosome primer/probe mix (BROWN)	165 µl

3. Prepare the Y chromosome detection mix according to the table below:

Component	1 reaction
PrecisionPLUS or PrecisionFAST or oasig Master Mix	10 µl
Y chromosome primer/probe mix (BROWN)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final volume	15 µl

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

5. 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).

6. 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 PrecisionPLUS or oasis 2X qPCR Master Mix

	Step	Time	Temp
	Enzyme activation	2min	95°C
X40 cycles	Denaturation	10s	95°C
	DATA COLLECTION*	60s	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
	Enzyme activation	2min	95°C
X40 cycles	Denaturation	5s	95°C
	DATA COLLECTION*	20s	60°C

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