YOUSEQ

HUMAN GENOMIC DNA QUANTIFICATION KIT USER GUIDE

CAT NO. YSL-qP-HugDNA-100

100 reactions
With lyophilised MasterMix

VERSION 2.0

For Research Use Only

YOUSEQ

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INTENDED USE

This product is a qPCR test kit for quantification of all human genomic material present in the reaction. Primers/probe are shown *in silico* to detect all publicly available human genome sequences and no other targets.

KIT CONTENTS

	Cap Colour	Volume
Target specific primer/probe (FAM Probe)		110 µl
Human gDNA Standard		100 µl *
Lyophilised Tetra 2X qPCR MasterMix		1.1 ml *
MasterMix Resuspension Buffer		1.5 ml
Template Resuspension Buffer		1.5 ml
DNase/RNase Free Water		1.5 ml

* Supplied lyophilised and requires resuspension, see resuspension step below for instructions

RESUSPENSION STEP

Resuspend the kit contents with the correct reagents as per the table below. Spin or gently tap all tubes and vials to ensure all the contents is at the bottom of the tube. After adding the resuspension reagent, pulse or vortex the vials again to ensure it is mixed well.

	Reagent	Volume
Human gDNA Standard	Template Resuspension Buffer	100 µl
Lyophilised Tetra 2X qPCR MasterMix	MasterMix Resuspension Buffer	1.1 ml

MATERIALS REQUIRED BUT NOT PROVIDED

Genomic DNA Extraction kit – This kit will work well with any DNA extraction kit that yields high quality DNA with minimal PCR inhibitors present.

qPCR instrument with minimum 1 colour detection (FAM).

Pipettes and general laboratory equipment.

qPCR BENCH SIDE PROTOCOL

Clean and decontaminate all work surfaces, pipettes, and other equipment prior to use to remove potentially contaminating the nucleic acids.

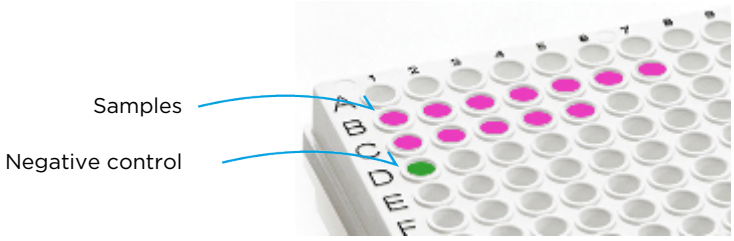
Combine the following reagents to create a final test reaction:

Component	Volume
Tetra 2X qPCR MasterMix	10 µl
Target specific primer/probe mix	1 µl
DNase/RNase Free Water	4 µl
Sample DNA	5 µl
Final Volume	20 µl

Please note: Set up your qPCR reaction plate on ice and proceed to amplification quickly. Prolonged incubation of the reaction mix, particularly at room temperature, can reduce the assay sensitivity.

NEGATIVE CONTROL

For a negative control reaction, repeat the reaction set up above replacing the sample DNA with RNase/DNase free water.



Please note: Make sure to seal the sample and negative control wells before proceeding to the positive control step.

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POSITIVE CONTROL STANDARDS

In your designated post-PCR environment, perform a serial dilution of the Human gDNA Standard to create a Six-point standard curve

a. Add 30 µl of template resuspension buffer into 5 tubes and label them 2, 3, 4, 5 and 6.
b. Pipette 10 µl of gDNA Standard into tube 2
c. Mix by pipetting up and down 5 times
d. Change pipette tip and pipette 10 µl from tube 2 into tube 3
e. Mix by pipetting up and down 5 times

Repeat steps 4 and 5 with the remaining tubes to complete the dilution process

Set up a reaction well for each point of the standard curve using the table below:

Component	Volume
2X qPCR MasterMix	10 µl
Target primer/probe mix	1 µl
DNase/RNase Free Water	4 µl
Selected dilution of gDNA Standard	5 µl
Final Volume	20 µl

The described standard curve provides a dynamic range as in the table below:

Tube No.	No. of pg/µL in PCR reaction
Tube 1	5,000
Tube 2	1,250
Tube 3	312
Tube 4	78
Tube 5	19
Tube 6	5

Positive control standards 1-6

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qPCR AMPLIFICATION PROTOCOL

This YouSeq kit will work with any qPCR instrument capable of detecting FAM. Use the following cycling conditions:

	Temperature	Time
	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds

*Data collection for appropriate target channels – FAM

INTERPRETATION OF RESULTS

gDNA Standards

Firstly, check the Standards performance. The first point of your standard curve should amplify in a Cq range of approximately 27+/-3. Amplification outside of this range suggests a failure and the test should be repeated.
To quantify your samples reliably, the standard curve must achieve an efficiency between 90% to 110%. This will be automatically calculated by your analysis software. If it falls out of this range, the run should be repeated with a freshly prepared standard curve.

Negative control

In ideal circumstances, the negative control well should deliver a flat line – negative result. However, it is not uncommon for background laboratory contamination to cause a very late signal. If this signal is ≥5 Cq values away from your sample signal, then it can be considered negative, and the result is viable.
If the negative control is <5 Cq away from your sample result, then the result is inconclusive, and the test should be repeated.

Positive samples

Samples that are positive for hugDNA will deliver a defined "sigmoidal" amplification plot. Your qPCR instrument software will calculate a quantitative result for these signals by comparing the signal to the positive control standard curve.

Results interpretation at a glance:

	qPCR Signal				
Human Samples	+	-	+/-	+/-	+/-
gDNA Standard	+	+	+/-	+	-
Negative control	-	-	≤33	>33	+/-
Result	Quantitative result	Negative result	Failed test (contamination)	See negative ctrl advice above	Failed Test

QUANTIFICATION OF SAMPLE GENOMIC DNA

Your qPCR instrument software will automatically compare the Cq values obtained from your samples to those from the Human gDNA standards in the kit. This calculation will deliver a 'calculated concentration' in ng/µl of each of your gDNA samples.

Refer to your qPCR instrument software for instructions.

If the sample concentration falls outside the dynamic range of the standards, dilute the sample, and try repeating the run.

PRODUCT SPECIFICATIONS

Storing your kit

Store at -20C. Kept frozen, the kit has a shelf life of 12 months.
Once you have prepared the Human gDNA Standard curve it can be stored frozen. However, if you observe a shift in Cq values in the standard curve over time a fresh standard curve should be prepared.

Use good quality gDNA

Poor quality input nucleic acid is the biggest cause of test failure. The kit will work well with any source of good quality gDNA. Good quality is defined as gDNA with high integrity (not degraded) and with low levels of inhibitors present.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each kit is tested against predetermined specifications to ensure consistent product quality.

Technical Assistance

For customer support, please contact:

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