

Primerdesign

R01391

Human Immunodeficiency Virus Types 1 & 2 and Hepatitis Viruses B and C

Kit version: 4

Target regions

HIV1: Gag gene

HIV2: 5'UTR

HBV: S gene

HCV: POLY gene

genesig[®]PLEX Kit

100 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Product Description

This genesig®PLEX qPCR kit is designed for the in vitro detection of human immunodeficiency virus types 1 and 2 (HIV1 and HIV2) and human hepatitis viruses B and C (HBV and HCV).

Individual primer and probes designed for each virus have been combined into a single reaction for a multiplex detection approach utilising different fluorescent channels.

HIV1 and HIV2 (FAM channel)

This genesig®PLEX qPCR detection kit targets the gag gene from HIV1 and the 5'UTR region from HIV2. HIV1 and HIV2 are single-stranded RNA viruses that infect humans. HIV1 is the predominant type globally, while HIV2 is less common and mainly found in West Africa. HIV2 generally shows slower disease progression and is associated with lower levels of transmissibility. Both viruses primarily infect CD4⁺ T lymphocytes and, over time, the loss and dysfunction of these cells weakens the immune system. If untreated, this can progress to AIDS (acquired immunodeficiency syndrome).

HBV (VIC channel)

This genesig®PLEX qPCR detection kit targets the S gene from HBV. HBV is a DNA virus which primarily infects humans and can also infect some non-human primates. HBV infection attacks the liver and can cause both acute and chronic disease with symptoms such as jaundice, fatigue, nausea, and abdominal pain. It can also progress to cirrhosis and liver cancer.

HCV (ROX channel)

This genesig®PLEX qPCR detection kit targets the POLY gene from HCV. HCV is an RNA virus which primarily infects humans, leading to inflammation of the liver, which can be asymptomatic or result in acute illness or chronic disease.

Specificity

This kit is designed for the in vitro detection of HIV1, HIV2, HBV and HCV and to have a broad detection profile.

HIV1 and HIV2

The HIV1 and HIV2 assays within this kit will detect over 95% of HIV1 and HIV2 sequences available on the Los Alamos National Laboratory database from the previous three years. Due to the sequence evolution observed in genomes of RNA viruses over time, this is considered to be an adequate period for analysis.

The HIV1 and HIV2 assays are both predicted to cross-react with Simian-Human immunodeficiency virus and Simian immunodeficiency virus.

HBV and HCV

The HBV and HCV assays within this kit are predicted to detect over 95% of sequences available on the NCBI database at the time of design or last review.

The HBV assay is specific for hepatitis B and is not predicted to cross-react with hepatitis A, C, D or E.

The HCV assay is specific for hepatitis C and is not predicted to cross-react with hepatitis A, B, D or E.

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	HIV1/HIV2/HBV/HCV V4.0 primer/probe mix (100 reactions) HIV1 (FAM) HIV2 (FAM) HBV (VIC) HCV (ROX) Endogenous control (Cy5)		BROWN
1	HIV1/HIV2/HBV/HCV V4.0 positive control template		RED
2	oasig® PLUS OneStep Lyophilised RT-qPCR Master Mix (50 reactions per glass vial)		GOLD
2	oasig® Master Mix resuspension buffer for resuspension of the lyophilised Master Mix		BLUE
1	Template preparation buffer for resuspension of positive control template		YELLOW
1	RNase/DNase free water for resuspension of the primer/probe mix, and negative control		WHITE

Reagents and equipment to be supplied by the user

Real-time PCR Instrument

Must be able to read fluorescence through FAM, HEX/VIC, ROX and Cy5.

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.

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/RNA integrity.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Principles of the test

Real-time PCR

Probe-based OneStep real-time PCR is a sensitive and specific detection method for RNA and DNA targets. During the initial reverse transcription step, RNA is converted into complementary DNA, which is subsequently amplified and measured during each PCR cycle. Target-specific primers hybridise to the target DNA and initiate amplification. A fluorogenic probe is included with the primer/probe mix, which consists of a target-specific oligo sequence labelled with a 5'-dye and 3'-quencher. When the target is present, the probe is cleaved during PCR amplification, and the reporter dye and quencher are separated, resulting in an increase in fluorescence that can be detected by the real-time PCR instrument.

This genesig®PLEX assay consists of specific primers and labelled probes for HIV1, HIV2, HBV and HCV, combined in a single reaction for a multiplex detection approach. The HIV1 and HIV2 are both detected in the FAM channel, HBV in VIC, HCV in ROX and the endogenous control in Cy5.

Positive control

The positive control contains templates for the three target channels: HIV1 and HIV2 will give a signal in FAM, HBV will give a signal in VIC and HCV will give a signal in ROX. 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 each virus are working properly in that particular run. 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 these components 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 or tube.

Negative control

It is recommended to include a negative control, also known as a No Template Control (NTC), in every run. For this, the RNase/DNase free water included in the kit should be used instead of a sample. A negative result indicates that the reagents have not become contaminated while setting up the run and thereby verifies any positive sample results.

Endogenous control

To confirm extraction of a valid biological template, the multiplex primer/probe mix supplied also contains primers and probe designed to detect an endogenous gene. Detection of the endogenous control is through the Cy5 channel. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Resuspension protocol

To minimise the risk of contamination with foreign DNA/RNA, 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 primer/probe mix 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	
HIV1/IHV2/HBV/HCV V4.0 Primer/probe mix (BROWN)	110 µl

3. Resuspend the positive control template in the template preparation buffer supplied, according to the table below:

To ensure complete resuspension, vortex the tube thoroughly.

Component - resuspend in template preparation buffer	Volume
Post-PCR heat-sealed foil	
HIV1/IHV2/HBV/HCV V4.0 Positive control template (RED) *	500 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.

4. Resuspend the oasig[®]PLUS OneStep Lyophilised RT-qPCR Master Mix in oasig[®] resuspension buffer, according to the table below:

Component - resuspend in oasig [®] resuspension buffer	Volume
oasig [®] PLUS OneStep Lyophilised RT-qPCR Master Mix (GOLD)	525 µl

RT-qPCR detection protocol

1. **For each extracted sample prepare a reaction mix according to the table below:**
Include sufficient reactions for all samples, positive and negative controls.

Component	Volume
oasig®PLUS OneStep Lyophilised RT-qPCR Master Mix (GOLD)	10 µl
HIV1/IHV2/HBV/HCV V4 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 experimental qPCR plate set-up.**
3. **Pipette 5 µl of extracted sample into each well according to your experimental plate set-up.**
For negative control wells use 5 µl of RNase/DNase free water (**WHITE**). The final volume in each well is 20 µl.
4. **Pipette 5µl of positive control template into the positive control wells according to your plate set-up.**
The positive control contains templates for HIV1, HIV2, HBV and HCV. The final volume in each well is 20 µl.

RT-qPCR amplification protocol

Amplification conditions for oasig[®]PLUS OneStep Lyophilised RT-qPCR Master Mix (**GOLD**):

	Step	Time	Temp
	Reverse transcription	10 mins	55 °C
	Enzyme activation	2 min	95 °C
Cycling x50	Denaturation	10 s	95 °C
	DATA COLLECTION *	60 s	60 °C

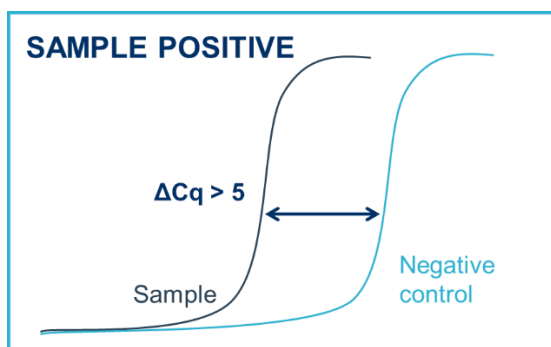
* Fluorogenic data should be collected during this step through the FAM, VIC, ROX and Cy5 channels.

As the HCV assay produces amplification through the ROX channel, please ensure ROX normalisation is switched off if using a qPCR instrument that recommends ROX as a passive reference dye.

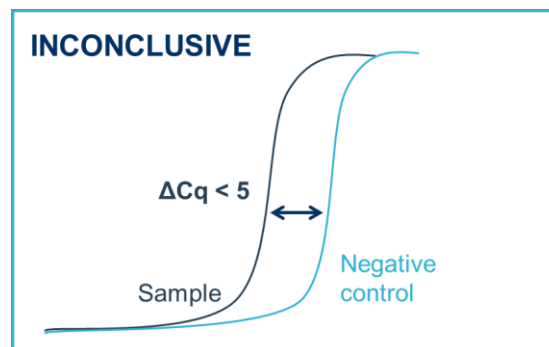
Interpretation of results

Target (FAM/VIC/ROX)	Endogenous control (CY5)	Positive control	Negative control	Interpretation
FAM +	+/-	+	-	HIV1/HIV2 POSITIVE RESULT
VIC +	+/-	+	-	HBV POSITIVE RESULT
ROX +	+/-	+	-	HCV POSITIVE RESULT
-	+	+	-	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 > 5 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 < 5 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.

Positive Control

The positive control well should produce amplification through the FAM channel (HIV1/HIV2), the VIC channel (HBV) and the ROX channel (HCV). There is no endogenous control template within the positive control so the Cy5 channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each virus.

The positive control is expected to amplify between Cq 16 – 23 in the FAM, VIC and ROX channels. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised and should be repeated.

Negative Control

The negative control is expected to be free of amplification. If the negative control shows amplification, please refer to the interpretation table for guidance, as this indicates contamination.

Endogenous control

The signal obtained from the endogenous control reaction will vary according to the amount of biological material present in each sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.

Sample data

Presence of the viruses are detected in the channels indicated in the kit contents section. Positive signals indicate positive tests for those viruses. It may be possible for samples to contain multiple viruses, therefore positive results in the FAM, VIC and ROX channels may be present.

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 in violation of the general GLP guidelines and the manufacturer's recommendations, the right to claim under guarantee is expired.

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