PathoPlex HIV, HBV, & HCV Qualitative RT-PCR Detection kit

20 Rxns | 2-30°C

cedbio

INTENDED USE

PathoPlex HIV, HBV, & HCV Qualitative RT-PCR Detection kit is a unique multiplex, qualitative RT-PCR assay for the specific detection of Human Immunodeficiency Virus (HIV), Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection in human plasma, serum and whole blood, along with a human internal control. The intended user of the product is medical/research professionals in laboratory, healthcare, etc.

SAMPLE TYPES

Human Whole Blood, Plasma, Serum

BACKGROUND

Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) are major global health concerns, impacting millions of people worldwide. These infections have significant public health implications and often coexist within affected individuals, leading to complex clinical scenarios and treatment challenges. Timely and accurate diagnosis of co-infections is critical for effective disease management, personalized treatment plans, and appropriate patient care. NeoDx's Pathoplex offers a breakthrough solution by combining the detection of HIV, HBV, and HCV in a single PCR reaction. This multiplex approach simplifies the testing process, enabling simultaneous identification of these viral infections from a single blood sample. By integrating multiple primer sets and probes specific to each virus into a single reaction, Pathoplex eliminates the need for multiple reactions, minimizing the risk of contamination, reducing hands-on time, and enhancing overall assay efficiency. Pathoplex represents a significant advancement in molecular diagnostics by providing a single-tube, multiplex RT-PCR solution for the simultaneous detection of HIV, HBV, and HCV. This innovative kit holds great promise in expediting the diagnosis of co-infections, enabling healthcare professionals to make timely and and informed decisions for effective patient management, improved treatment outcomes, and the overall control of these viral infections.

PRODUCT DESCRIPTION

PathoPlex HIV, HBV, & HCV Qualitative RT-PCR Detection kit is an *in vitro* diagnostic kit which detects HIV (HIV-1 & HIV-2), HBV and HCV present in the infected persons sample using RT-PCR. Reverse transcriptase present in the master mix converts the HIV and HCV RNA into cDNA. The converted cDNA and HBV DNA will be amplified using the TaqHS with the help of specific primers and simultaneous detection is achieved by the fluorescently labeled probes along with human internal control.

MATERIALS PROVIDED

KIT COMPONENTS	VOLUME
Master Mix	300 µL
Primer and Probe Mix	30 µL
Nuclease Free Water	100 µL
Positive Test Control (PTC)	20 µL
Instruction for Use	1 No

MATERIALS REQUIRED BUT NOT PROVIDED

Consumables

PCR Plates/tubes

PCR plate covers/tube caps

COMPATIBLE INSTRUMENTS

Real-Time PCR, instruments like, BIORAD-CFX96, THERMO -QS5, QIAGEN-ROTOR - GENE Q and other instruments which supports which supports Cy5 (649 nm - 670 nm), FAM (495 nm - 520 nm), HEX (535 nm - 556 nm) and Texas Red (596 nm - 615 nm).

TARGET	REPORTER	QUENCHER
HIV-1 & HIV-2	Cy5	BHQ2
HBV	FAM	BHQ1
HCV	HEX/VIC	BHQ2
Human Internal Control	TEXAS RED/ROX	BHQ2

***Note:** In some instruments where the BHQ option is unavailable, please set the quencher to "NONE."*

STORAGE AND HANDLING

- Store all PathoPlex HIV, HBV, & HCV Qualitative RT-PCR Detection Kit reagents at -20°C.

- Do not repeatedly freeze-thaw reagents more than five times as it leads to reduced assay sensitivity. Thaw the reagents only on ice or at 4° C. Recommended freeze thaw cycle time is 5 times.
- Kit components are stable through the end of the expiration date indicated on the box when stored at -20°C. Shelf Life - 12 Months from date of manufacture.

PRECAUTIONS

- It is recommended that this product is used by personnel specially instructed and trained in real-time PCR and *in-vitro* diagnostics procedures.
- Treat all the specimens as potentially infectious.
- Wear protective disposable powder-free gloves, a laboratory coat & eye protection when handling specimens.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Keep separate areas for master mix and template preparation and work under biosafety cabinets.
- Use aerosol barrier pipette tips.
- Do not open the reaction tubes/plates post-amplification, to avoid amplicon contamination.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Do not use kit components that have passed their expiration date.

REACTION MIXTURE - 35 µL

Reagents	1 Rxn	20 Rxn
Master Mix	15 µL	300 µL
Primer and Probe mix	1.5 µL	30 µL
Nuclease Free Water	3.5 µL	70 µL
Total	20 µL	400 µL

Add 15 µL of the test RNA/DNA per reaction

SAMPLE PREPARATION

RNA/DNA should be extracted from freshly collected whole blood, plasma or serum aspirates using any approved nucleic acid extraction methods. Store the extracted RNA/DNA at - 20 °C for further use.

TEST PREPARATION/REACTION SET-UP

- Thaw all components of the kit on ice, mix gently using vortex and spindown the contents for 5 sec and use it immediately.
- Calculate the number of reactions for each experiment including all controls with one excess reaction volume in the reaction cocktail to accommodate pipetting errors. (eg: number of reaction (n) including controls are 10 add 1 extra reaction during the preparation n+1) .
- Prepare the reaction mix in a 1.5/2 mL tube for the calculated number of samples in Master Mix Preparation room.
- Spin down the tubes and dispense 20 µL reaction mix in each tube strips or 96 well plate. Before moving to template adding area, add 15 µL of nuclease free water in NTC wells.
- Carefully add 15 µL samples kept on ice in the designated wells in template addition room. Add 2 µL of PTC in a separate hood and make up the volume by adding 13 µL of nuclease free water.
- The assay should be run along with positive controls and negative controls.
- Seal the plate carefully, briefly spin down and use any qRT-PCR instrument which complies with the dyes specified in the kit insert.

THERMAL CYCLING CONDITION

STEP	TEMP °C	TIME	DETECTION	CYCLE
Reverse Transcription	45	20 Min	Off	1
	95	2 Min	Off	1
PCR and Detection	95	15 Sec	Off	40
	58	30 Sec	On	

READING TEST RESULTS / DATA ANALYSIS

- NTCs should be negative and should not exhibit fluorescence amplified curves that cross the threshold line.
- If a false positive occurs with one or more of the primer and probe in NTC (Negative Test Control) reactions, it indicates sample contamination.
- In that case, Invalidate the run and repeat the assay with stricter adherence to the procedure guidelines.
- Positive control should produce a positive result with an expected cycle threshold (Ct/Cq) value for each target included in the test.

- If expected positive reactivity is not achieved, invalidate the run and repeat the assay with stricter adherence to procedure guidelines.
- After completion of the run, analyze the data as per the instrument manufacturer instructions.
- Analysis should be performed separately for each target using a manual threshold settings.
- In case internal control has not worked for a sample re-do the test with 2 or more dilutions.
- Negative results do not exclude possibility of infection and should not be used as the sole basis for the treatment.

RESULTS INTERPRETATION

For HIV, HBV, HCV	For Internal Control	Assay Result
Ct < 37	Ct ≤ 37	Positive
Ct = Undetermined or Ct ≥ 37	Ct ≤ 37	Negative
Ct = Undetermined or Ct ≥ 37	Ct ≥ 37 Undetermined	Invalid. Re-purify the nucleic acid from the sample, then repeat the test.

DATA INTERPRETATION

HIV-1 & HIV-2 CY5	HBV FAM	HCV HEX/ VIC	IC Texas Red/ROX	RESULTS INTERPRETATION
+	-	-	+	Human Immunodeficiency Virus (HIV-1 & HIV-2) is detected
-	+	-	+	Human Hepatitis Virus B is detected
-	-	+	+	Hepatitis Virus C is detected
-	-	-	+	Negative for HIV, HBV & HCV infection.
-	-	-	-	Invalid results repeat the RNA extraction and re-run

ANALYTICAL SPECIFICATION

Performance evaluation of PathoPlex HIV, HBV and HCV Qualitative RT-PCR Detection kit is traceable to WHO International standard 4th WHO International Standard for HIV-1 RNA (NIBSC code: 16/194), 2nd WHO International Standard for HIV-2 RNA for Nucleic Acid Amplification Techniques (NIBSC code:16/296) 4th WHO International Standard for HBV DNA for NAT NIBSC code: 10/266 & 6th WHO International Standard for Hepatitis C virus RNA (NIBSC code:18/184).
Limit of detection of HIV is 20 IU/mL, HBV is 32 IU/mL and HCV is 85 IU/mL for ≥95% of the time.

CLINICAL PERFORMANCE

		Approved Predicate Kit		
		Positive	Negative	Total
PathoPlex HIV, HBV, & HCV Qualitative RT-PCR Detection kit	Positive	19	1	20
	Negative	1	19	20
	Total	20	20	40

Parameter	Estimate	95% CI
Sensitivity	95%	75.13 to 99.87%
Specificity	95%	75.13 to 99.87%

WASTE DISPOSAL

- Dispose all the waste/remains of the reagents used in reaction mixture preparation & expired kit components along with bio-waste as per the lab manual/general bio-waste management instruction.
- Dispose the PCR plates with patient samples "sealed" post run to avoid potential infection to the operators and contamination of the lab.

TROUBLESHOOTING

Positive control showed no amplification

Cause - Inappropriate storage of reagents
- Store the reagents at recommended temperature for their optimal performance.
- Avoid repetitive freezing and thawing.
- Check the expiry of reagents.

Negative controls are positive

Cause - Cross-contamination
- Follow good laboratory practices to avoid contamination issues.
- Use a new batch of reagents and repeat the experiment.

Abnormal plot and/or low ΔRn values in amplification curve

Cause - The baseline was set improperly (some samples have CT values lower than the baseline value)
- Switch from manual to automatic baseline, or move the baseline stop value to a lower CT (2 cycles before the amplification curve for the sample which crosses the threshold).

An amplification signal is detected in the early cycles

- Dilute the sample to increase the CT value.