ViPrimePLUS Dirofilaria immitis qPCR Kit

Product Code: VICD001
Pack Size: 100 reactions

INTENDED USE

The ViPrimePLUS Dirofilaria immitis qPCR Kit is a Taqman probe-based real-time PCR assay for the detection and quantification of *Dirofilaria immitis* (D.immit) DNA in good quality nucleic acid samples from a variety of sources. This assay is intended for the research use and trained users in molecular biology laboratory environment.

INTRODUCTION

ViPrimePLUS Dirofilaria immitis qPCR Kit has designed to have very high homology with all reference data within the NCBI database. The kit has the broadest detection profile and detects all clinically relevant strains. The kit detects Dirofilaria immitis. The kit is also known as Dog heartworm nematode.

PRINCIPLE OF TEST

The kit contains the target specific primers and Taqman® probe, and this can be detected through the FAM channel. The ROX dye provided allows the kit to be used on all range of qPCR platforms.

The kit provides the Internal Extraction Control (IEC) as inhibition control. During nucleic acid extraction protocol, IEC DNA template is added in the lysis stage. An IEC specific primers and probe provided to be run in the same reaction and the IEC amplicons are detected via VIC/HEX channel.

A positive control with known copy number is provided for standard curve construction and absolute quantification. It can also be used at a single dilution for qualitative analysis control of the experimental set-up.

QUALITY CONTROL

Each lot of ViPrimePLUS Dirofilaria immitis qPCR Kit has been tested against predetermined specifications to ensure consistent product quality under ISO 9001:2015 – certified Quality Management System.

SENSITIVITY & SPECIFICITY

The detection limit is tested to have at least 100 copies per reaction. The primers and probe are 100% specific.

STORAGE & STABILITY

Store at -20ºC upon arrival and avoid light exposure. Stable at -20°C up to the expiry date stated. Keep in aliquot to reduce freeze-thaw cycles. Always store the kit at -20°C once the lyophilized tube has reconstituted. Ship and store at ambient temperature not more than 10 days.

LIMITATION OF TEST

For research use only. Not recommended for diagnosis of disease in humans or animals.
Result is dependent on the yield and quality of the nucleic acids extracted from the method of extraction. Thus, it is important to do spectrophotometric and gel analysis on the extracted samples.

KIT COMPONENTS

Kit Components	Tube Colour	Volume (ml)
Dirofilaria immitis Primers and Probe Mix (gene PPM – FAM probe)	Amber	0.1 ml
Positive Control Template	Pink	Lyophilized
Internal Extraction Control Primers and Probe Mix (IEC PPM – HEX probe)	Blue	0.1 ml
Internal Extraction Control Template (IEC)	Red	Lyophilized
2X PCR Master Mix	Purple	Lyophilized
ROX Reference Dye	Black	0.01 ml
Nuclease-free Water	White	1.5 ml
Template Preparation Buffer	Orange	1.5 ml
Master Mix Preparation Buffer	Transparent	1.5 ml

PROTOCOL 1

Reconstitution of Reagents

*Pulse-spin each tube prior to opening.

Components	Volume	Reagents
Positive Control Template	500 µl	Template Preparation Buffer
Internal Extraction Control Template	500 µl	Template Preparation Buffer
2X PCR Master Mix	1.1 ml	Master Mix Preparation Buffer

PROTOCOL 2

DNA extraction

Add **5 µl** of IEC Template into each sample suspended in the lysis/extraction buffer.

**__DO NOT__ add IEC directly into unprocessed biological sample to avoid DNA degradation*

Real-time PCR Platform ROX Dependent Check and ROX Tube Preparation

ROX Reference Dye is required for some real-time PCR platform as passive reference dye.
Check the real-time PCR platform and prepare the ROX Reference Dye accordingly.

ROX Types	Real-time PCR Platform	Volume of Nuclease-free Water added to ROX Tube
High ROX	Applied Biosystems 7000, 7300, 7700, 7900 and 7900HT FAST platforms, OpenArray, PRISM 7000, 7700, 7900, GeneAmp® 5700, StepOne™, StepOne™ PLUS; Roche Lightcycler® series	No required
Middle ROX	Agilent / Stratagene Mx3000, Mx3005P, Mx4000, Mx4000R	75 µl
Low ROX	Applied Biosystems 7500 and 7500 FAST platform, QuantStudio™, ViiA7.	130 µl
No ROX	All other real-time PCR platform	No required

For the real-time PCR platform that requires ROX Reference Dye, after dilution done according to table above, add **5 µl** to the reconstituted 2X Master Mix.

Real-time PCR reaction set-up

Recommended real-time PCR reaction set-up:

Reagents	1 reaction (µl)
2X PCR Master Mix	10
Gene Primers and Probe Mix	1
IEC Primers and Probe Mix (not required when preparing standards' reaction mix)	1
Sample DNA	5 – 8
Nuclease-free Water	Top up to 20 µl

**Suggested sample concentration 5-100ng/µl.*
**Adjust Nuclease-free Water to make up the final reaction volume of 20 µl.*
**Prepare an extra reaction to accommodate for pipetting error.*

Negative control

Set aside one tube/well as negative control where 8 µl of Nuclease-free Water is used as the template. This is also known as no template control.

Positive control

For qualitative analysis, set aside one tube as positive control where 5 µl of the positive control is used as the template

Positive control (optional)

For quantitative analysis only, prepare 6 serially diluted positive control templates for standard curve construction.

a) Pipette 90 µl of Nuclease-free Water into 5 clean microtubes and label 2-6 accordingly.
b) Pipette 10 µl of Positive Control into tube 2.
c) Vortex thoroughly and spin down.
d) Change pipette tip and pipette 10 µl from tube 2 to tube 3. Vortex and spin down.
e) Repeat steps d) to complete the dilution series.
f) Pipette **5 µl of standard template** into each tube/well accordingly.

Standards	Copy number/µl
Tube 1 Positive Control	1 x 10^6
Tube 2	1 x 10^5
Tube 3	1 x 10^4
Tube 4	1 x 10^3
Tube 5	100
Tube 6	10

PROGRAM

Set the thermal cycler parameters as follows:

Step	Time	Temp	Cycles	Scan
Enzyme activation	3 mins	95°C		
Denaturation	15 secs	95°C	45	
Anneal/Elongation	1 min	60°C		√*

*Make sure to collect fluorogenic data through FAM and HEX channels during this step

INTERPRETATION OF RESULTS

Pathogen specific amplification signal is detected via FAM channel, while IEC amplification is detected via VIC/HEX channel. The signal is positive if the amplification curve crosses the threshold line. The result is relevant provided both positive and negative controls give valid results.

When analysing sample Ct values, the threshold is checked within the run file before interpreting the data. The suggested threshold is set to 10% of the relevant positive control End Point Fluorescence.

Summary of interpretation:

Target (FAM channel)	IEC (HEX channel)	Negative Control	Positive Control (FAM channel)	Interpretation
+; ≤35	+; ≤31	-	+; ≤21	Valid, positive
+; ≤35	-	-	+; ≤21	Valid, positive
-	+; ≤31	-	+; ≤21	Valid, negative
-	-	-	-	Invalid
+; ≤35	+; ≤31	+	+; ≤21	Invalid

Positive Control

When used accordingly and assuming between 90% to 110% efficiency, the undiluted positive control should amplify in a Ct value of 18±3. If running a positive control standard curve for a quantitative result, an efficiency between 90% to 110% will provide most accurate quantification. If both fall outside of the range, the repeated test is suggested to be repeated.

Internal Extraction Control

When used accordingly and assuming 100% extraction efficiency, a Ct value of 28±3 is within normal range with the indication of a good extraction. A high Dirofilaria immitis genome copy amplification may out compete the IEC amplification. Thus, the latter may not produce an amplification signal. The positive result is still valid in this case.

Negative Control

The negative control should not have Ct value and achieve flat line. However, it is not uncommon for laboratory contamination to have very late signal. If the negative control is having ≥5 Ct values later than the sample signal, then it can be considered negative and the sample result can be considered as valid. If the negative control is ≤5 Ct values, the result is considered as invalid.

Positive Samples

The cut off Ct value of the samples is ≤35; suggested to have defined “sigmoidal” amplification plot delivered. In order to present the results in target copy number, a quantitative result and signal can be calculated by comparing the signal to the positive control standard curve.

WARRANTY AND LIMITED LIABILITY

The performance characteristics stated were obtained using the assay procedure in this insert. Failure to comply with the instructions may derive inaccurate results. In such event, manufacturer disclaims all warranty expressed, implied or statutory including the implied warranty of merchantability and the fitness of use.

The manufacturer will not be liable for any damage caused by misuse, improper handling and storage; non-compliance with precautions and procedures, and damages caused by events occurring after the product is released.