



February 2025

Quick-Start Protocol

Pv/Pm/Po/Pk Detection Assay

The Pv/Pm/Po/Pk Detection Assay kit (cat. no. 225113) is intended for molecular biology application for human epidemiological research using qPCR. Upon receipt the kit should be stored at -30 to -15°C in a constant-temperature freezer and protected from light.

Further information

- QIAprep& Plasmidium Kit Handbook: www.qiagen.com/HB-3663
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com
- Pf/Non-Pf Detection Assay Kit Quick-Start Protocol: www.qiagen.com/HB-3669

Notes before starting

- This assay allows species identification of samples positive for non-falciparum Plasmodium detected by the Pf/Non-Pf Detection Assay (cat. no. 224113). The assay is compatible with all qPCR cyclers with the mentioned dye detection capabilities.
- Each tube contains primers and probes for detection of *Plasmodium vivax* (*P. vivax*), *Plasmodium malariae* (*P. malariae*), *Plasmodium ovale* (*P. ovale*), and *Plasmodium knowlesi* (*P. knowlesi*) (Pv/Pm/Po/Pk).

- The *P. ovale* signal can be detected in FAM;
 - The *P. vivax* signal can be detected in HEX;
 - The *P. malariae* signal can be detected in Cy5; and
 - The *P. knowlesi* signal can be detected in Cy5.5.
- Each tube of the Pv/Pm/Po/Pk Detection Assay contains a mixture of primers and probes at a 20 x concentration; each tube contains sufficient reagents for 100 reactions (100 µL).

Procedure

1. Ensure that the qPCR cyclers has the following detection channels: FAM, HEX, ROX, Cy5, and Cy5.5.
2. Follow the reaction setup and cycling condition of the QIAprep& Plasmodium Kit when using this assay. Refer to www.qiagen.com/HB-3663
3. Select the relevant detection channels (FAM, HEX, Cy5, and Cy5.5) in the PCR cycler during run setup. See Table 1 for alternative channel names.

Table 1. Dyes, typical channel names and their alternative names

Dye	Alternative channel names in qPCR cyclers	
	QIAquant	RGQ
FAM	Blue channel	Green channel
HEX/JOE/VIC	Green channel	Yellow channel
Cy5	Red channel	Red channel
Cy5.5/Quasar705	NIR1	Crimson

Result interpretation

Table 2. Result interpretation in the different channels from analyzed human samples

Signal in FAM (<i>P. ovale</i>)	Signal in HEX (<i>P. vivax</i>)	Signal in Cy5 (<i>P. malariae</i>)	Signal in Cy5.5 (<i>P. knowlesi</i>)	Status	Result
+	–	–	–	VALID	Positive signal for Po
–	+	–	–	VALID	Positive signal for Pv
–	–	+	–	VALID	Positive signal for Pm
–	–	–	+	VALID	Positive signal for Pk
–	–	–	–	INVALID	Repeat test

Note: Positive signals in more than one channel can indicate mixed infections.

Note: The Pv/Pm/Po/Pk Detection Assay is designated to be used after the Pf/Non-Pf Assay (cat. no. 224113) in cases when a signal for Non-Pf was recorded. Therefore the Pv/Pm/Po/Pk Detection Assay should show a signal for at least one of the species. If no signal is observed, then the test is invalid and needs to be repeated.

Document Revision History

Date	Changes
02/2025	Initial release



Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

Trademarks: QIAGEN®, Sample to Insight®, QIAcard®, QIAprep&®, Uni-Core® (QIAGEN Group); Whatman® (Whatman International Limited Corporation). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

02/2025 HB-3671-001 © 2025 QIAGEN, all rights reserved.