February 2025

Quick-Start Protocol

Pf/Non-Pf Detection Assay

The Pf/Non-Pf Detection Assay kit (cat. no. 224113) 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&amp Plasmodium Kit Handbook*: **www.qiagen.com/HB-3663**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**
- *Pv/Pm/Po/Pk Detection Assay Kit Quick-Start Protocol*: **www.qiagen.com/HB-3671**

Notes before starting

- The assay is compatible with all qPCR cyclers with the mentioned dye detection capabilities.
  Each tube contains primers and probes for detection of *Plasmodium falciparum* (*P. falciparum*) and *Plasmodium spp.*. Additionally, an assay for the detection of human RNase P is included as sampling and in-process PCR control.
  - The *P. falciparum* signal can be detected in the FAM channel;
  - Other Plasmodium species (*Plasmodium spp.*) can be detected in the Cy5 channel; and

- The signal for RNase P can be detected in the HEX channel.

- Each tube of the Pf/Non-Pf Detection Assay Kit contains a mixture of primers and probes at a 20x concentration; therefore, only 1 µL of the assay is needed per 20 µL reaction. Each tube contains sufficient reagents for 100 reactions.

Procedure

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

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

	Alternative channel names in qPCR cyclers	
Dye	**QIAquant**	**RGQ**
FAM	Blue channel	Green channel
HEX/JOE/VIC	Green channel	Yellow channel
Cy5	Red channel	Red channel

Result interpretation

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

Signal in FAM* (P. falciparum)	Signal in HEX† (In-process control)	Signal in Cy5‡ (Non falciparum)	Status	Result
+	+	–	**VALID**	Positive signal for Pf
–	+	+	**VALID**	Positive signal for Non-Pf
+	–	–	**VALID**	Positive signal for Pf
–	–	+	**VALID**	Positive signal for Non-Pf
+	+	+	**VALID**	Sample with a mixed infection
+	–	+	**VALID**	Sample with a mixed infection
–	+	–	**VALID**	Negative signal for Plasmodium
–	–	–	**INVALID**	Repeat test

* A positive signal in the FAM channel indicates the presence of Plasmodium falciparum in the analyzed human sample

†A signal in the HEX channel indicates that the PCR reaction was successful and human sample material was present in the reaction.

‡Non-falciparum species: *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium knowlesi*, and/or *Plasmodium malariae*.

Note:If no signal is observed, then the test is invalid and needs to be repeated.

Summary of the *in silico* investigation on potential cross-reactive species

Due to the close relationship among different Plasmodium species, the possibility of cross-reactivity was investigated. The following species might be detected by the assays based on an *in silico* analysis (Table 3 and Table 4):

Table 3. Detected species in Plasmodium falciparum assay

Plasmodium sp. gorilla
Plasmodium sp. chimpanzee
Plasmodium billcollinsi
Plasmodium lutzi

Table 4. Detected species in Non-falciparum assay

Plasmodium brasilianum	*Plasmodium gonderi*	*Polychromophilus murinus*	*Plasmodium minuoviride*
Plasmodium inui	*Plasmodium sp. chimpanzee*	*Leucocytozoon caulleryi*	*Plasmodium azurophilum*
Plasmodium cynomolgi	*Plasmodium sp. gorilla*	*Polychromophilus melanipherus*	*Haemoproteus ptyodactylii*
Plasmodium fragile	*Plasmodium hylobati*	*Plasmodium juxtanucleare*	*Plasmodium elongatum*
Plasmodium simium	*Plasmodium simiovale*	*Plasmodium leucocytica*	*Plasmodium billcollinsi*
Plasmodium fieldi	*Plasmodium coatneyi*	*Plasmodium circumflexum*	*Plasmodium lacertiliae*
Plasmodium sp. pongo	*Haemosporida*	*Plasmodium megalotrypa*	*Leucocytozoon sp.*
Hepatocystis sp.	*Polychromophilus sp.*	*Plasmodium koreafense*	*Plasmodium reichenowi*

Document Revision History

Date	Changes
02/2025	Initial release