



February 2025

Quick-Start Protocol

QIAprep&® Plasmodium Kit Whole Blood Workflow

The QIAprep& Plasmodium Kit (cat. no. 223213) should be stored immediately upon receipt at -30 to -15°C in a constant-temperature freezer and protected from light. Several components of the kit can be stored at room temperature (15 – 25°C): Blood Lysis Buffer, PR Buffer, DBS Wash Buffer. The S-Solution is stored light-protected at -20°C . This kit can be used for the detection of the Plasmodium parasite (in combination with the respective assay) in different workflows. The components used differ between workflows.

This workflow is designated for whole blood; it is intended to be used with whole blood samples collected in EDTA, citrate or heparin tubes.

Further information

- QIAprep& Plasmodium 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
- Pv/Pm/Po/Pk Detection Assay Kit Quick-Start Protocol: www.qiagen.com/HB-3671

Notes before starting

- Thaw the QP&A DNA Mastermix, the PCR assay of choice, and RNase-free water.
- Additionally, this protocol uses PR Buffer and S-Solution.
 - The PR buffer forms a precipitate upon storage below 15°C. If necessary, redissolve by mild agitation at 37°C and then place at room temperature.
- Use the cycling conditions specified in this protocol.

Procedure

1. Vortex and centrifuge all reagents and each blood sample.
2. Pre-dispense 2 µL of PR Buffer into each PCR tube or well of a PCR plate.
3. Transfer 4 µL of blood to the tube or well of the PCR plate. Mix by pipetting up and down, or vortex for 5–10 s. Make sure to add the blood to the PR Buffer and not vice versa.
4. **Important:** Seal the plate/tube thoroughly to prevent cross-contamination. If an adhesive film is used, make sure to apply pressure uniformly across the entire plate, to obtain a tight seal across individual wells. Centrifuge it briefly to collect the liquid at the bottom of the plate/tube.
5. Incubate for 5 min at 95°C in a PCR Cycler (with heated lid). After incubation, let the samples cool down to room temperature.

Note: The blood starts clotting during the heating step. This is normal and expected.

6. Centrifuge the plate/tube briefly.
7. **PCR setup:** Prepare the PCR reaction mix for a multiplex PCR reaction as shown in Table 1. Vortex briefly and centrifuge.

Table 1. Reaction mix setup

Component	Channel for detection	1 rxn (µL)	Final Concentration
QP&A DNA Mastermix	–	9	1x
20x Assay Mix	Select respective channels	1	1x
S-Solution	–	2	–
RNase-free Water	–	2	–
Total volume	–	14	–

8. Remove the cover of the plate/tube and add 14 µL of PCR reaction mix (Table 1) to each well.
9. Seal the plate/tube thoroughly with a fresh foil/lid. Mix gently by vortexing with medium pressure (5–10 s). Place the plate in different positions while vortexing, to ensure an equal contact with the vortex platform.
10. **Important:** In the heating step (step 5) the blood can curdle a bit. It is perfectly normal if some wells do not show a perfect mixture of blood and PCR reaction mix. This does not affect performance.
11. Centrifuge the plate/tube briefly to collect the liquid at the bottom of the plate/tube. Place it in the real-time cyclers and start the cycling program (with heated lid). Program the cycler as referred to in Table 2.

Note: Data acquisition should be performed during the annealing/extension step.

Table 2. Cycling conditions

Step	Time	Temperature (°C)	Ramp rate
PCR initial heat activation	2 min	95	Maximal/fast mode
2-step cycling (40 cycles)			
Denaturation	5 s	95	Maximal/fast mode
Combined annealing/extension*	30 s	58	Maximal/fast mode

*Add data acquisition

Result interpretation

Refer to the following when using these assay kits:

- Pf/Non-Pf Detection Assay Kit Quick-Start Protocol*: www.qiagen.com/HB-3669
- Pv/Pm/Po/Pk Detection Assay Kit Quick-Start Protocol*: www.qiagen.com/HB-3671

For general qPCR result interpretation, refer to the *QIAprep& Plasmodium Kit Handbook* at www.qiagen.com/HB-3663

Document Revision History

Date	Changes
02/2025	Initial release

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

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