February 2025

Quick-Start Protocol

# QIAprep&amp® Plasmodium Kit Sedimentation Workflow

The QIAprep&amp 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 ultrasensitive detection of Plasmodium from whole blood; it is intended to be used with whole blood samples collected in EDTA, citrate or heparin tubes.

## Further information

- *QIAprep&amp 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 Blood Lysis Buffer, 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 400 µL Blood Lysis Buffer into a new tube (e.g. 1.5–2 mL tube)
3. Add 40 µL blood to the tube. Vortex for 5 s.
4. Put the tubes into a centrifuge with the hinges facing the exterior, this will help in ensuring that the sediment forms at the same spot in every tube. Centrifuge for 2 min at 4300 x *g*.

   **Note**: After centrifugation, a small pellet may become visible at the bottom of the tube. If the pellet is very small it may not be visible.
5. Carefully remove and discard the supernatant completely using a pipette. Do not decant the supernatant.
6. Add 30 µL PR Buffer to the pellet and incubate for 10 min at room temperature.
7. Pipet up and down thoroughly 10–20 times to resuspend the pellet.
8. Transfer 4 µL of dissolved pellet to a new tube or well of the PCR reaction plate and seal the tube/plate.

   **Important**: Seal the plate/tube thoroughly to prevent cross-contamination. In case 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.

**Note**: Keep the remaining dissolved pellet (e.g. for a reflex test). It is recommended to store the dissolved pellet at –20°C for up to 1 month. The dissolved pellet can be subjected up to 8 freeze–thaw cycles.

9. Incubate for 5 min at 95°C in a PCR cycler (with heated lid). After incubation, let the samples cool down to room temperature. Centrifuge the plate/tube briefly.

10. **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 | – | 4 | – |
| **Total volume** | **–** | **16** | **–** |

11. Remove the cover of the plate/tube and add 16 µL of PCR reaction mix (Table 1) to each well.

12. 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.

13. Centrifuge the plate/tube briefly to collect the liquid at the bottom of the plate/tube. Place it in the real-time cycler 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:

a. *Pf/Non-Pf Detection Assay Kit Quick-Start Protocol*: **www.qiagen.com/HB-3669**

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

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

## Document Revision History

| Date | Changes |
|---|---|
| 02/2025 | Initial release |