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BIOTOOLS B&M LABS.S.A.

BIOMALAR *GEL FORM KIT*

REF.	FORMAT	CONTENTS
90.001	48 rxns	BIOMALAR *GEL FORM KIT*

Store at 4ºC

Warning for users: Some of the applications that can be performed with this product are protected by patents applicable in some countries. Purchase of this product does not include or provide a licence to perform patented applications. In some cases, depending on the country and/or application, users are required to purchase a licence.

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1. DESCRIPTION

The **Biomalar *Gel Form* kit** allows the qualitative detection of *Plasmodium* species in clinical samples. Detection involves two steps: the first step detects the presence of DNA from the protozoan *Plasmodium sp.* and the second identifies the *Plasmodium* species present in the sample. This kit allows identification of the four main species of this parasite responsible for malaria in humans (*P. falciparum, P. malariae, P. ovale* and *P. vivax*).

The parasite DNA present in the sample is specifically amplified using primers that hybridise with homologous sequences from the *Plasmodium* genome. The **Biomalar *Gel Form* kit** is a fast and easy method in which the two amplification reactions are supplied in a "ready to use" gelified format. This gel format reduces handling times and the risk of contamination without compromising the efficacy or sensitivity of the assay.

2. REAGENTS INCLUDED WITH THE KIT

The **Biomalar *Gel Form* kit** contains sufficient reagents for 48 assays. Kit contents:

- ***Gelified Plasmodium Mixture:*** Gelified reaction mixture that allows amplification of the genus *Plasmodium sp.* This reaction includes a pair of human primers as an internal amplification control, thereby allowing the presence of PCR inhibitors in the amplification reaction to be ruled out. The kit contains 6 strips of 8 transparent gelified tubes (48 reactions).
- ***Gelified Species Mixture:*** A gelified reaction mixture that allows specific amplification of the species *P. falciparum, P. malariae, P. ovale* and *P. vivax.* The kit contains 6 strips of 8 coloured gelified tubes (48 reactions).
- ***P. falciparum Positive Control:*** Gelified amplification product. The kit contains 1 gelified vial (*yellow label*).
- ***P. malariae Positive Control:*** Gelified amplification product. The kit contains 1 gelified vial (*red label*).
- ***P. ovale Positive Control:*** Gelified amplification product. The kit contains 1 gelified vial (*blue label*).
- ***P. vivax Positive Control:*** Gelified amplification product. The kit contains 1 gelified vial (*green label*).

Both mixtures (*Plasmodium* and *Species)* include BIOTOOLS DNA Polymerase, reaction buffer, $MgCl_2$, dNTPs, primers and stabilisers in their formulation in gelified format. To perform the amplification reactions, you only need to add the template DNA (or the DNA from the positive controls) and nuclease-free water.

3. HANDLING AND STORAGE

Upon receipt, store all components of the Biomalar *Gel Form* Kit at **4ºC**. The kit may be shipped and handled at room temperature.

The ***gelified positive controls*** must be reconstituted prior to use. Once rehydrated, these controls must be stored as aliquots at -20°C in order to prevent them from being submitted to freeze/thaw cycles. These aliquots must be thawed and stored in ice for subsequent handling.

If handled and stored according to these recommendations, the stability of this kit will be as indicated on the corresponding label.

4. SOURCE MATERIALS

The **Biomalar *Gel Form* kit** can be used with the following source samples:

- *Whole blood*
- *Whole blood with anticoagulants* (heparin, EDTA, sodium citrate, etc.)
- *Blood deposited on a filter paper or card*

The use of commercial column extraction kits (e.g. Speedtools DNA Extraction kit) or magnetic beads is recommended for purification of parasite DNA.

5. PREPARATION OF POSITIVE CONTROLS

Always work in a specific area for the handling of positive DNA controls. This area must be separated from other working zones, especially the extraction and purification zone or the reagent-preparation zone.

Note: *Doubly distilled, sterile, nuclease-free water must be used to prepare the positive controls.*

1.- Dilute one vial of each positive control in **500 µL** Tris 10 mM pH 8.0 (or nuclease-free water) and mix on a vortex for a short period of time (15 sec).

2.- Prepare aliquots of each resuspended control and store at -20 °C until use. Once thawed, the control aliquots should not be refrozen.

6. INSTRUCTIONS FOR USE

I. Plasmodium Amplification

1.- Calculate the number of reactions required, including at least one negative control (free from DNA). Select the appropriate number of ***Gelified Plasmodium Mixture*** gelified tube strips.

2.- Add 37.7 µL sterile nuclease-free water to each positive reaction vial. Negative controls (free from DNA) should be made up with 42.7 µL sterile nuclease-free water.

3.- Add 5 µL template DNA (50-100 ng) to each positive sample vial.

Note 1: *The contents of the gel do not need to be resuspended; the gelified reagents will be resuspended during the initial denaturation step.*

4.- Program the thermal cycler according to the guidelines in Table 1. Place the vials in the thermal cycler and run the amplification program selected.

Table 1. PCR1 Program

STEPS	N° CYCLES	TEMPERATURE	TIME
Initial denaturation	1	94ºC	7 min
Denaturation Annealing Extension	45	94ºC 62ºC 72ºC	20 sec 20 sec 30 sec
Final Extension	1	72ºC	10 min
Cooling	∞	4ºC	∞

5.- Store the products from the first amplification reaction at 4°C.

Note 2: *The positive controls for the various Plasmodium species are included in the second PCR step, during Species Amplification.*

II. Species Amplification

1.- Put aside the appropriate number of ***Gelified Species Mixture*** gelified tubes (one vial for each *Plasmodium Amplification* reaction + one vial for each positive control included in the kit).

2.- Thaw one aliquot of each reconstituted positive control and store in ice (see point 5).

3.- Add 19.2 µL sterile nuclease-free water + **2 µL of the product amplified** in the *Plasmodium* Amplification step to each vial.

4.- Add 16.2 µL sterile nuclease-free water + **5 µL of the corresponding reconstituted positive control** to the positive control vials.

Note 3: *The contents of the gel do not need to be resuspended; the gelified reagents will be resuspended during the initial denaturation step.*

5.- Program the thermal cycler according to the guidelines in Table 2. Place the vials in the thermal cycler and run the amplification program selected.

Table 2. PCR2 Program

STEPS	N° CYCLES	TEMPERATURE	TIME
Initial denaturation	1	94ºC	5 min
Denaturation Annealing Extension	35	94ºC 53ºC 72ºC	15 sec 90 sec 20 sec
Final Extension	1	72ºC	10 min
Cooling	∞	4ºC	∞

6.- Store the products from the second amplification reaction at 4°C.

III. ELECTROPHORESIS

The amplification products are analysed by horizontal electrophoresis on agarose gels and visualised using DNA intercalating agents (e.g. ethidium bromide, SYBR® Green).

1.- Prepare 2% agarose gels

2.- Load 10 µL of the products from both amplification reactions onto the agarose gel.

3.- Include lanes with suitable molecular weight markers (e.g. 100 bp Marker). Migrate the electrophoresis

4.- Check the amplification products obtained (see point 7).

7. INTERPRETING THE RESULTS

I. Plasmodium Amplification

One or two amplification bands (231 and 783 bp) will be seen in this first amplification reaction. The band at **231 bp**, which corresponds to the human **internal control**, will always appear (except for negative controls) provided no amplification inhibitors are present in the reaction.

The higher **783 bp** band, which corresponds to the ***Plasmodium sp***. DNA fragment, will be seen in patients with high parasitaemia.

II. Species Amplification

In the event of *Plasmodium sp*. infection, the specific band for the parasite species responsible for the disease will be seen in the second amplification reaction.

Similarly, the band corresponding to the control included will be seen in the amplification products for the positive controls.

The band pattern for each of the various *Plasmodium* species detected by the kit is as follows:

- *P. malariae:* **241 bp** amplicon
- *P. falciparum:* **370 bp** amplicon
- *P. ovale:* **407 bp** amplicon
- *P. vivax:* **476 bp** amplicon

8. TROUBLESHOOTING

Low or null reaction efficiency

1. **Check the quantity and quality of the template.** Check the integrity of the DNA by electrophoresis on denaturing agarose gel and the quantity of template by fluorimetry (an excess of DNA may decrease the yield of the amplification reactions).
   An excess of some purification reagents may interfere with PCR: reduce the volume of template or change the purification method. Ensure that the materials used are nuclease-free.
2. **Check the shelf-life and storage conditions of the kit.** Check that the expiry date indicated on the outer label has not been exceeded. Check for the absence of hydration in the gelified tubes.
3. **Ensure suitable resuspension of the gels.** In the event of suspected incomplete resuspension of the gels during the initial denaturation step of the PCR, the process can be aided by mixing the reaction mixture on a vortex prior to placing it in the thermal cycler (centrifuge after mixing).
4. **Check the PCR program.** Check that the correct amplification programs have been selected for both reactions.
5. **A reaction component is missing.** Check that the DNA template has been included in the *Plasmodium Amplification* step.
6. **Absence of the 231 bp band in the *Plasmodium Amplification* step.** Presence of PCR inhibitors in the PCR. Purify the DNA template again, or dilute it and repeat the assay.

Multiple and/or non-specific amplification products

1. **Check the quantity and quality of the template.** Check the integrity and concentration of the DNA. Reduce the quantity of template to be included in the *Plasmodium Amplification* step.
2. **Check the PCR program.** Check that the amplification programs selected for both reactions are appropriate.
3. **Rule out contamination of the reaction water with DNA.** In the event of contamination of the negative control vials (DNA-free), change the batch of water used.