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MYCOPLASMA *GEL DETECTION* KIT

Pre-aliquoted kit for the detection of mycoplasma in cell culture samples by endpoint PCR

REF.	FORMATO	CONTENIDO
90.021	48 rxns	Mycoplasma *Gel Detection Kit*
90.022	192 rxns	Mycoplasma *Gel Detection Kit*
90.044	1 vial	Gelified Positive Control
90.045	1 vial	Gelified Internal Amplification Control

Store at -20ºC

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.

Ed. 06- August 2023

1. DESCRIPTION

Mycoplasmas, extremely small and simple bacteria from the *Mollicutes* group, are the main contaminants in cell cultures. Although barely perceptible, *Mycoplasma* may have serious effects on eukaryotic cells, altering normal gene expression, immunity and cell growth. The contamination of cell cultures with *Mycoplasma* therefore represents a serious problem in basic research and biopharmaceutical manufacturing.

The **Mycoplasma *Gel Detection* kit** from Biotools allows *Mycoplasma* contamination in cell culture supernatants to be detected by endpoint PCR by amplification of a conserved region of 16S rRNA. The Biotools kit simultaneously detects an internal amplification control, thereby allowing the presence of PCR inhibitors in the sample can be identified.

The Mycoplasma *Gel Detection* kit is a fast, easy and sensitive method in which the amplification reaction is supplied in a ready to use gelified format. This pre-aliquoted gel format reduces handling times and the risk of contamination, and allows handling at room temperature without compromising the efficacy or sensitivity of the assay.

The Mycoplasma Gel Detection kit identifies more than 20 of the species most commonly found infecting cell cultures, including *M. arginini, M. fermentans, M. hyorhinis, M. orale, A. laidlawi* (see list in the Appendix). The kit does not detect other prokaryotic or eukaryotic DNA templates. The sensitivity of the PCR using the Biotools kit is about 10 template DNA copies per reaction.

2. KIT COMPONENTS

The **Mycoplasma *Gel Detection* kit** contains sufficient reagents for 48 assays. Kit contents:

- ***Gelified Mix:*** 6 Strips of 8 gelified vials (48 reactions), or 24 strips of 8 gelified vials (192 reactions).
  Gelified and pre-aliquoted reaction mixture optimised for a final volume of 25 µl, includes all reaction components, including the primers. In addition to the specific primers for *Mycoplasma spp.* detection, the gelified mix includes a pair of primers for amplification of the internal control, which allows the presence of PCR inhibitors in the reaction mixture can be identified.
- ***Gelified Positive Control (PC):*** 1 Gelified vial (*red label*) (48 reactions) or 2 Gelified vials (red label) (192 reactions). Fragment of non-infectious *M. fermentans* DNA prepared by PCR and gelified.
- ***Gelified Internal Amplification Control (IAC):*** 1 Gelified vial (*blue label*) (48 reactions) or 2 Gelified Vials (blue label) (192 reactions) Unrelated non-infectious DNA fragment with a specific sequence prepared by PCR and gelified.

The **Gelified Mix** includes DNA Polymerase, reaction buffer, $MgCl_2$, dNTPs, primers and stabilisers in its formulation in gelified format. To perform the amplification reactions, you only need to add the template DNA (or DNA from PC), IAC DNA and nuclease-free water to a total volume of 25 µL.

3. HANDLING AND STORAGE

Upon receipt, store all components of the Mycoplasma *Gel Detection* Kit at **-20ºC**. The kit can be shipped at room temperature.

The ***gelified controls*** must be reconstituted prior to use. Once rehydrated, they must be stored as aliquots at **-20°C** in order to prevent them from being submitted to freeze/thaw cycles. It is recommended to make the aliquots in low retention tubes. 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. INSTRUCTIONS FOR USE

A. Sample preparation and handling

The **Mycoplasma Gel Detection kit** has been optimised for use with samples of cell culture supernatant. For correct sample collection, the cell lines must be cultured to 90-100% confluence in the absence of antibiotics specific for *Mycoplasma*. The medium sample should be collected 48-72 h after the last medium change in order to increase assay sensitivity.

Once the cell culture supernatant (500-1000 µL) has been collected in a sterile vial, the sample should be handled according to one of the protocols described below:

I. Standard Protocol: *Heat inactivation of DNases*

If the sample is not processed immediately, inactivate the DNases by thermal inactivation. These enzymes are able to act even at low temperatures, degrading the template DNA.

1- Incubate the supernatant at 95 °C for 10 min (use screw-capped vials or strengthen the stopper seal).
2- Briefly centrifuge (10-30 sec) to remove cell debris prior to pipetting the sample.
3- Store the samples at 4 °C for 3 days. For longer storage times, store at -20 °C.

II. Alternative Protocol: *Centrifugation-based enrichment*

This protocol allows sample enrichment and increases the sensitivity of the assay. In addition, it removes the possibility of interference due to components of the culture medium.

1- Centrifuge at 13,000 x g for 10-15 min (precipitate not always visible).
2- Discard the supernatant and add 50 µL Tris-HCl 10 mM (or nuclease-free water) to the precipitate, mixing on a vortex.
3- Heat-inactivate the DNases by incubating the supernatant at 95 °C for 5 min (use screw-capped vials or strengthen the stopper seal).
4- Store the samples at 4 °C for 3 days. For longer storage times, store at -20 °C.
5- Centrifuge for 10-30 sec to remove cell debris prior to pipetting the sample.

B. Preparation of Gelified Controls

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

1- Dilute the **IAC** and **PC** vials in 500 µL Tris 10 mM pH 8.0 (or nuclease-free water) by pipetting up and down and gently mix on a vortex.

2- Prepare aliquots of each resuspended control and store at -20 °C until use. It is recommended to make the aliquots in low retention tubes.

3- Once thawed, the aliquots should not be refrozen. These aliquots must only be used once in order to avoid DNA degradation.

C. Reaction Mixture Preparation

1- Calculate the number of reactions required, including at least one negative control (free from DNA) and one positive control. Set aside the appropriate number of **Gelified Mix** vials.

2- Add the various reaction components according to the instructions in Table 1.

Table 1. ***Reaction Mixture Preparation***

COMPONENT	Control (-)	Control (+)	Samples
IAC DNA *(blue label)*			6ul
PC DNA *(red label)*		5µl	
Sample			2µl
Nuclease-free water	25µl	20µl	17µl

Note: *Centrifuge the sample before removing an aliquot. When pipetting ensures that no precipitate is collected as* ***cell debris contains a high content of*** *PCR inhibitors.*

3- **Do not resuspend the gels**, the gelified reagents will be resuspended during the initial denaturation step of the amplification program. Gently mix manually (do not use a vortex), then gently centrifuge.

4- Program the thermal cycler according to the instructions in Table 2, place the vials in the thermal cycler and start the amplification program.

Table 2. ***Amplification Program***

N° Cycles	Temperature	Time
1	95°C	2 min
32	95°C 58°C 72°C	15 seg 90 seg 30 seg
1	72°C	5 min
	4-8°C	∞

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

5- Prepare a 2% agarose gel.

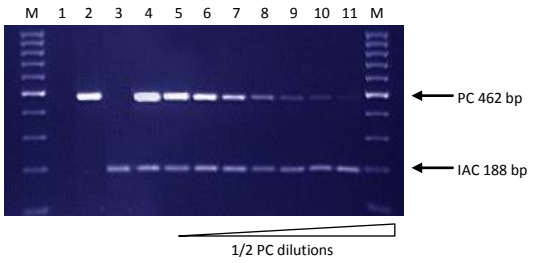
6- Load 14 µl of the reaction product mixed with the loading buffer. Include lanes with suitable molecular weight markers.

7- Migrate the electrophoresis and verify the amplification products obtained.

Expected band pattern:

- **PC** band: 462 bp
- **IAC** band: 188 bp
- *Mycoplasma spp.* band: 435-470 bp (depending on the species present in the sample)

Figure 1. ***Band Pattern***



M lanes: 100 bp DNA Ladder
Lane 1: PCR negative control (DNA-free)
Lane 2: PC
Lane 3: IAC
Lanes 4-11: IAC + 1/2 PC dilutions ($1x10^3$-7.8 copies/reaction)

D. Results interpretation

Sample	Bands visualised		Interpretation
	435-470 bp	188 bp	
Sample + IAC	NO	NO	False negative: inhibitor in sample or PCR not valid
	NO	**YES**	Absence of Mycoplasma (*Mollicutes) in sample*
	YES	NO	High Mycoplasma *(Mollicutes)* content in sample prevents visualisation of IAC
	YES	**YES**	Presence of Mycoplasma *(Mollicutes)*
PC + IAC	NO	NO	PCR not valid
	NO	**YES**	Problem with PC
	YES	**NO**	Problem with IAC
	YES	**YES**	Expected band pattern
IAC	NO	NO	PCR not valid
	NO	**YES**	Expected band pattern
	YES	NO	False positive: contamination and problem with IAC
	YES	**YES**	False positive: contamination
Negative control (ADN-free)	NO	NO	Expected result
	NO	**YES**	False positive: contamination
	YES	NO	False positive: contamination
	YES	**YES**	False positive: contamination

5. TROUBLESHOOTING

Absence of amplification in PC

1. **Control DNA degraded.** Use a new aliquot of resuspended PC. Remember that, once resuspended, the gelified PC should be stored in aliquots at -20 °C in order to prevent deterioration upon freezing/thawing. It is recommended to make the aliquots in low retention tubes
2. **Pipetting error.** Check that the PC has been added to the reaction vial.
3. **Check the thermal cycler program.** Check that the correct amplification program has been selected.
4. **Gelified mix vials hydrated.** Check for the absence of hydration in the Gelified Mix vials.

Absence of specific and IAC bands in the sample

1. **Presence of amplification inhibitor in the sample.** Dilute the sample with Tris 10 mM pH 8.0 or nuclease-free water and repeat the assay. If the quantity of inhibitors is significant, the template DNA can be extracted to prevent interference.
2. **IAC DNA degraded.** Use a new aliquot of resuspended IAC. Once resuspended, the gelified IAC should be stored in aliquots at -20 °C in order to prevent deterioration upon freezing/thawing. It is recommended to make the aliquots in low retention tubes
3. **Check the PCR program.** Check that the correct amplification program has been selected.
4. **Gelified mix vials hydrated.** Check for the absence of hydration in the Gelified Mix vials.

Appearance of a *Micoplasma spp.* band in the IAC vial

1. **IAC DNA contaminated.** Repeat the assay with a new aliquot of IAC; if contamination persists, resuspend a new gelified IAC vial.
2. **Reaction water contaminated.** Repeat the assay with a new aliquot of nuclease-free water.

Lack of reproducibility for sample duplicates

1. **Sample not homogeneous.** Centrifuge the sample prior to pipetting and repeat the assay. Do not touch the precipitate, which is enriched with PCR inhibitors, when pipetting. After centrifuging, the supernatant can also be transferred to a clean vial.

6. APPENDIX

Main *Mollicutes* species detected by the **Mycoplasma *Gel Detection* Kit**:

A. laidlawii
M. agalactiae
M. arginini
M. arthritidis
M. bovis
M. falconis
M. fermentans
M. hominis
M. hyorhinis
M. opalescens
M. orale
M. pirum
M. pneumoniae
M. pulmonis
M. salivarium
M. spermatophilum
M. synoviae
M. timone
Spiroplasmas
Ureaplasmas