MycoStrip™ 50 | 100

Mycoplasma detection kit

Catalog code: rep-mysnc-50, rep-mysnc-100

https://www.invivogen.com/mycostrip

For research use only, not for clinical use

Version 22L15-NJ

Contents

	50 Tests	*100 Tests*
• Reaction Mix	5 vials	10 vials
• Reaction Buffer	4 vials	8 vials
• Migration Buffer	1 bottle	2 bottles
• Positive Control	1 vial	2 vials
• Detection strips	50	100

Storage and stability

- Kit is shipped at room temperature.
- Store all components (incl. the detection strips) at -20°C.
- Stable for 12 months when stored properly.

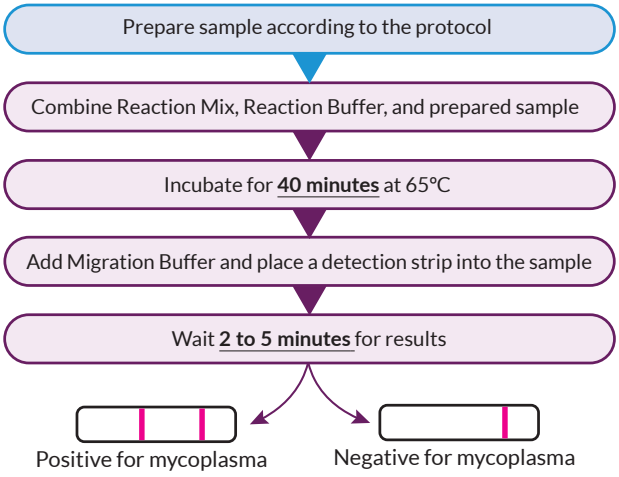
Other materials and consumables required

- Thermocycler (recommended) or heat block
- Pipettes with corresponding filter tips (recommended)
- 0.2 ml and 1.5 ml microcentrifuge tubes
- Bench centrifuge
- Sterile phosphate buffered saline (PBS)

TEST PRINCIPLE

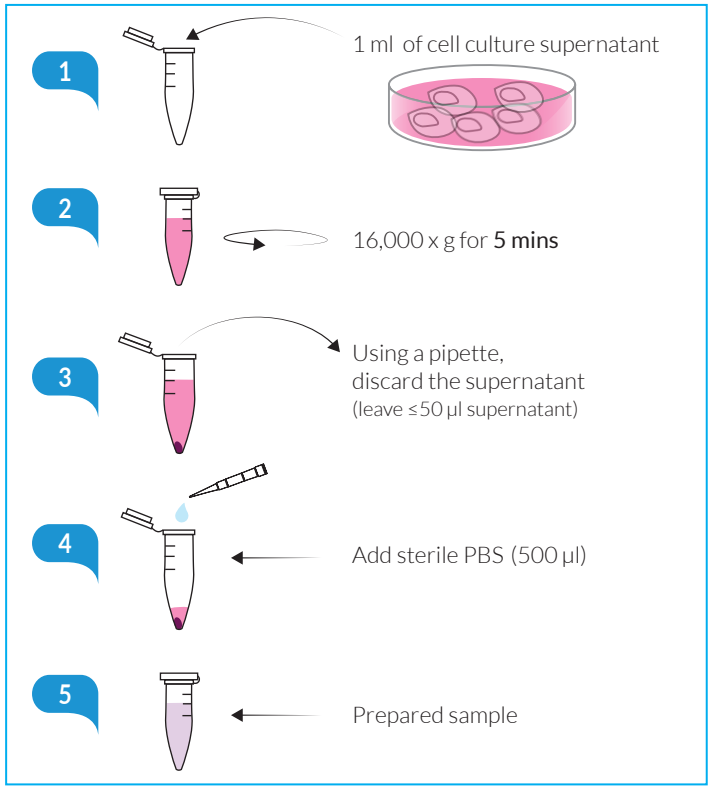
Detection of cell culture contaminating mycoplasma by MycoStrip™ is based on isothermal PCR. The 16S rRNA gene for the most commonly found mycoplasma species in cell culture, accounting for 95% of contaminations, is targeted and amplified using our proprietary Reaction Mix. Results are visualized as a band on an immunochromatographic strip within 5 minutes.

MycoStrip™ Protocol Outline



PREPARATION OF SAMPLES

MycoStrip™ can be used to test cell culture supernatant from suspension or adherent cell cultures prior to passaging, or fetal bovine serum (FBS).



METHOD

Caution: In preparation of cell culture samples, you may be handling live mycoplasma. Handle samples with due care and attention.

1. Transfer 1 ml of the cell culture supernatant to a microcentrifuge tube.
Note: The supernatant can be stored short term at 4°C (one week) or long term at -20°C. Before use, mix well by vortex.
2. Centrifuge at 16,000 x *g* (RCF) for 5 minutes to pellet the mycoplasma.
Note: The pellet will be invisible except if some cells have been collected. Importantly, their presence will not affect the detection assay.
3. Using a pipette, carefully discard the supernatant without disturbing the pellet. Ensure there is no more than 50 µl of medium remaining, as it will impact the detection assay.
4. Add 500 µl of sterile PBS and mix well by pipetting.
Note: For increased sensitivity, concentrate the sample 10x by repeating steps 2-3. Afterwards, proceed directly to step 5.
(Optional) - Heat the sample at 95°C for 5 minutes using a heat block to kill the mycoplasma.
5. Use prepared sample immediately or store at -20°C until required.

Positive & negative controls (optional use)

- *A 'ready-to-use' positive control is provided with the kit. It is NOT a source of mycoplasma contamination, it only contains DNA.*
- *A negative control is not included in the kit. Use sterile PBS.*

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873 / info@invivogen.com
InvivoGen USA (International): +1 (858) 457-5873
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MycoStrip™ DETECTION ASSAY

GENERAL RECOMMENDATIONS

To avoid cross contamination, we recommend to use filter tips throughout the protocol and to change tips between tubes at each step.

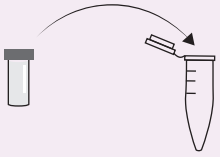
BEFORE STARTING THE ASSAY

- With every use, equilibrate all samples and reagents to room temperature.
- **Upon first use:**
  - **Spin down and gently homogenize (by flicking) 1 vial of the Reaction Mix.**
  - **Prepare 10 x 15 µl aliquots**, plus 1 or 2 extra aliquots.
  - Use aliquots immediately or store at -20°C until required.
- **IMPORTANT:** before each use, mix well the **Reaction Buffer** by **several vortex pulses** to ensure no precipitation of salt.
- Set the thermocycler or heat block to 65°C.

Before starting the assay

Make 15 µl aliquots of the Reaction Mix (1 vial)

(Use immediately or store at -20°C)



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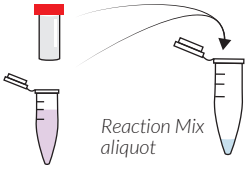
- Mix well Reaction Buffer by several vortex pulses
- Add Reaction Buffer (5 µl)
- Add prepared sample or control (5 µl)

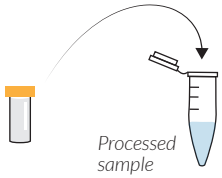


DO NOT VORTEX

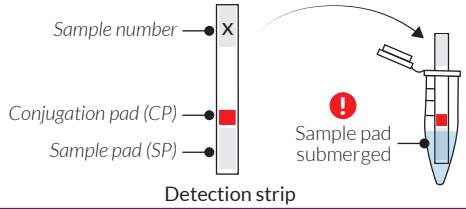
2 Incubate at **65°C** for **40 mins**

DO NOT EXCEED 65°C and 40 mins

3 Add Migration Buffer (200 µl) to the processed sample tube and mix well



4 Place the detection strip into the processed sample tube



METHOD

1. In a 15 µl Reaction Mix aliquot add first
   - 5 µl of the Reaction Buffer, then
   - 5 µl of the prepared sample (or control), and gently mix.

DO NOT VORTEX as this inhibits the reaction.

(Optional) - Include a negative control (not provided) and/or a positive control (provided). If you are including a negative and positive control, prepare them before and after your sample, respectively, to reduce any cross-contamination.

2. Incubate the reaction tube at **65°C** for **40 mins** using a thermocycler.

Caution: If using a heat block, check the temperature with a thermometer.

DO NOT EXCEED 65°C and 40 mins as this significantly impacts the reaction.

3. Briefly spin sample down (e.g. pulse spin for 5 sec.), and immediately add 200 µl of the Migration Buffer to stop the reaction. Mix well.

4. Place a detection strip directly into the processed sample with the **conjugation pad (CP) end in the tube**. Ensure that the sample pad (SP) is submerged. Wait 2 to 5 minutes for the revelation of results.

Caution: If the detection strip is placed into the processed sample in the wrong direction the revelation will not work. Discard this strip and use another.

Note: The colour of the conjugation pad will disappear after the test is performed.

Note: The processed sample can be stored at -20°C for up to 3 months.

DATA INTERPRETATION

There are two distinct results using MycoStrip™:

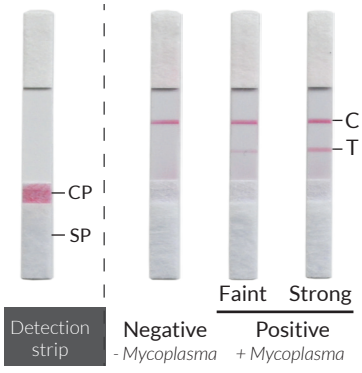
- **One band ('C' band only)**

Indicates that mycoplasma was not detected in the processed sample.

- **Two bands ('C' and 'T' band)**

Indicates that the processed sample is contaminated by mycoplasma.

Note: The migration control band ('C') will appear within 1-2 minutes. If after 5 minutes there is no test band ('T'), the test is negative.



- **To confirm a 'faint' positive result**: concentrate your sample or continue to grow the culture for an additional 48 hours. To concentrate your sample, start with a larger volume of supernatant and/or repeat steps 2-3 in the "preparation of samples" protocol (*other side*). Additionally, we recommend to use sterile water instead of PBS for the sample resuspension in step 4. Re-test the newly prepared sample using MycoStrip™.
- **If your sample is positive:** eradicate your contamination by treating your culture for 2 weeks using InvivoGen's Plasmocin™ or Plasmocure™.

For more information visit: www.invivogen.com/mycoplasma-elimination

SENSITIVITY & SPECIFICITY

MycoStrip™ has been specifically designed to detect the Mycoplasma and Acholeplasma species that most commonly contaminate cell cultures. These include the six species that account for 95% of all contaminations: *M. orale*, *M. hyorhinis*, *M. arginini*, *M. fermentans*, *M. hominis*, and *A. laidlawii*. Importantly, no cross-reactivity with any other tested bacterial, fungal, or mammalian DNA was observed with MycoStrip™.

MycoStrip™ can be used to detect mycoplasma contamination before it significantly affects experimental results, which typically occurs at ~10^7 CFU/ml. MycoStrip™ is able to detect as low as 10-10^2 CFU/ml, allowing effective treatment of the cells with anti-microbial agents.

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