MycoQuant™

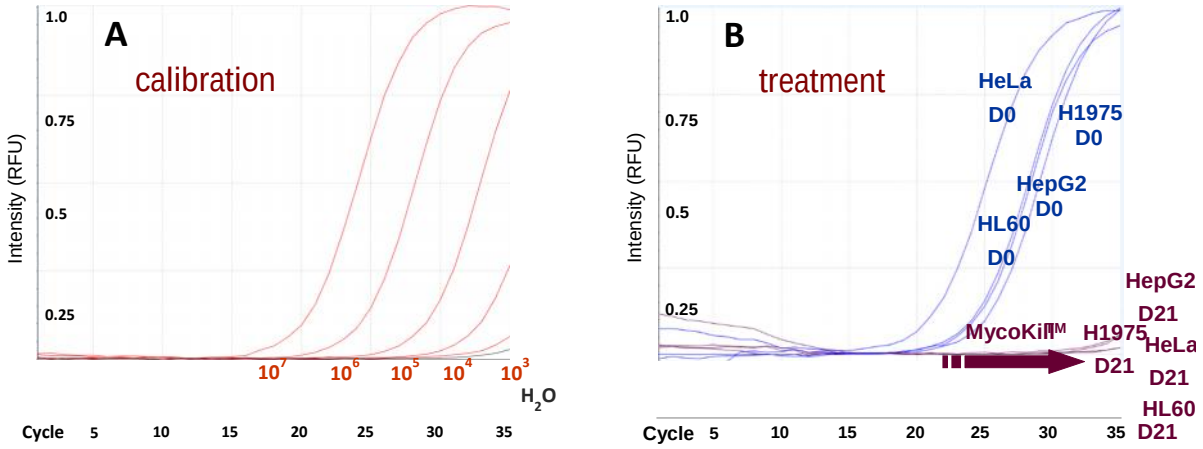
Mycoplasma Quantification Kit

MycoQuant™ (Cat# MQ-050, MQ-100, MQ-250) is used detect mycoplasma and related cell wall-less bacteria infection from cells.

In cell culturing mycoplasma contamination is a major problem regarding both research and industrial production. Unlike fungal or bacterial infections, mycoplasmal contaminations cannot be detected by visual inspection, but can lead to unreliable experimental results and potential unsafe biological products.

MycoQuant™ is an easy-to-use, multi-platform detection method, performed on any Real-Time PCR instrument.

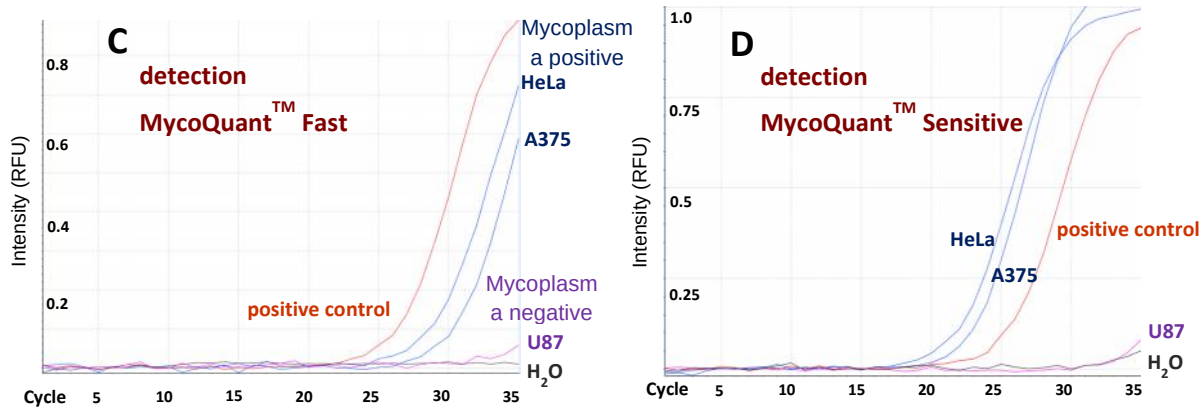
The protocoll is very simple and fast, the user only needs 1 reagent mix to add the templates.



In case of detecting mycoplasma infection, **MycoKill™** (Avidin, Cat# MK-2L, MK-8L) treatment is suggested, which eliminates the infection only in three weeks (Fig. B).

With this kit the user can perform two types of tests depending on the demand of sensitivity.

1) **MycoQuant™ Fast:** Detecting infection from cell culture supernatant (250 µl), fastest way of detection.
2) **MycoQuant™ Sensitive:** Using 20-100 ng of isolated genomic DNA, more sophisticated test.



Running the tests can identify not only the presence of mycoplasma infection, but the extent of infection can also be quantified by comparing the samples to the positive control included in the kit (10^6 DNA copies/reaction).

Warning: Avidin's anti-mycoplasma products are suitable for research purposes only, and not for human or animal care.

Technical Data Sheet

Section 1 - Product and Company Information

Product Name: **MycoQuant™**
Cat. Code: **MQ-050, MQ-100, MQ-250**
Company identification: Avidin Ltd., H-6726 Alsó kikötő sor 11.
Szeged, Hungary
+36 62/202-107

Section 2 – PRODUCT INFORMATION

Content:
- **MQ-050**: MycoQuant™ PCR reagent kit for 50 reactions, provided in a paper box containing 1x 0.625 ml 2x Reaction Mix solution; 1x 0.125 ml 10x Primer Mix solution; 1x 0.125 ml 2x Positive Control solution; 1x 0.125 ml 2x Negative Control solution; 1x 0.5 ml 2x Fast Preparation buffer.

- **MQ-100**: MycoQuant™ PCR reagent kit for 100 reactions, provided in two paper boxes each containing 1x 0.625 ml 2x Reaction Mix solution; 1x 0.125 ml 10x Primer Mix solution; 1x 0.125 ml 2x Positive Control solution; 1x 0.125 ml 2x Negative Control solution; 1x 0.5 ml 2x Fast Preparation buffer.

- **MQ-250:** MycoQuant™ PCR reagent kit for 250 reactions, provided in five paper boxes each containing 1x 0.625 ml 2x Reaction Mix solution; 1x 0.125 ml 10x Primer Mix solution; 1x 0.125 ml 2x Positive Control solution; 1x 0.125 ml 2x Negative Control solution; 1x 0.5 ml 2x Fast Preparation buffer.

Shipping and Storage:
- MycoQuant™ is shipped on dry ice and should be stored at -20 °C.
- MycoQuant™ is stable for 6 months at -20 °C.
- Avoid repeated freeze-thaw cycles.

The kit must be stored at -20°C and protected from sunlight. Under these conditions, the kit can be used until the expiration date marked on the labels. An inappropriate storage can affect reagents quality.

Quality Control:
Activity of each lot of MycoQuant™ kit is controlled by QPCR tests.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Section 3 – GENERAL PRODUCT USE

MycoQuant™ utilizes quantitative real-time PCR protocol to detect mycoplasma and related cell wall-less bacteria contamination in mammalian cell cultures. MycoQuant™ contains a Taqman-based assay set with FAM™ probe specific for mycoplasma sequences and an internal positive control for assay function set with VIC® - TAMRA™ probe. Genomic DNA purified from cells or cell culture supernatants, or a simple suspension, prepared from debris centrifuged from cell culture supernatant resuspended in a special buffer of the kit (2x Fast Preparation buffer), is added to a 2x Mastermix containing all ingredients for PCR. After cycling on a Real-Time PCR instrument (Roche, Thermo, ABI, BioRad, RotorGene, Bioneer, etc.) positive ampification curve in the FAM channel represents positive mycoplasma infection. Use a calibrated thermocycler for the fluorescence channel reading FAM™, VIC® or HEX™ or equivalent. The kit does not contain ROX™.

Section 4 – BACKGROUND

Mycoplasma are notorious contaminants of cell culture and contamination is a major problem in both industrial applications and basic research. Up to 60% of cell lines may be contaminated by mycoplasma[1,2]. Mycoplasma cannot be detected by visual inspection, however it often negatively affect cell growth rates. Moreover, mycoplasma infection has been shown to alter DNA synthesis, RNA and protein expression, induce chromosomal aberrations and introduce alterations or modifications of host cell plasma membrane proteins, including antigenes leading altered immune response[3]. Mycoplasmas can have profound effects on host cell biology by depriving cells of nutrients and inducing global changes in gene expression[4].

1. Cell culture contamination: an overview. Langdon SP. Methods Mol Med. 2004;88:309-17. Review.
2. Beware of mycoplasmas. Rottem S, Barile MF. Trends Biotechnol. 1993 Apr;11(4):143-51. Review.
3. Induced expression of melittin, an antimicrobial peptide, inhibits infection by Chlamydia trachomatis and Mycoplasma hominis in a HeLa cell line. Lazarev VN, Parfenova TM, Gularyan SK, Misyurina OY, Akopian TA, Govorun VM. Int J Antimicrob Agents. 2002 Feb;19(2):133-7.
4. Assessing the prevalence of mycoplasma contamination in cell culture via a survey of NCBI's RNA-seq archive. Olarerin-George AO, Hogenesch JB. Nucleic Acids Res. 2015 Mar 11;43(5):2535-42.

Section 5 – DESCRIPTION

MycoQuant™ is a mycoplasma PCR Detection Kit utilizes PCR, which is well established as the protocol of choice for highest sensitivity in the detection of mycoplasma contamination in cell cultures and other cell culture derived biological products. The kit is a one-step Taqman-based assay to detect mycoplasma and related cell wall-less bacteria infection from cells (set with FAM™ probe) including an internal positive control for assay function (set with VIC® - TAMRA™ probe). An internal control probe and the internal control DNA is already included in the Primer/Probe/ Nucleotide Mix. This probe emits fluorescent light with amplification of the Internal Control DNA with VIC/HEX signal confirming mycoplasma negative, but successfully performed test reactions (no inhibitors, accurate pipetting). The primer/probe reagent set detects the highly conserved 16S rRNA operon coding region of the mycoplasma genome. The kit is highly specific to this region and does not detect eukaryotic DNA. The detection spectrum includes most mycoplasma species identified as cell culture contaminants; see the following list of species:

M. agalactiae M. agassizii M. alligatoris M. alkalescens M. anatis M. anseris M. arginini M. arthritidis M. bovigenitalium M. bovirhinis M. bovis M. bovoculi M. buccale M. buteonis M. californicum M. canadense M. canis M. capricolum M. caviae M. citelli M. cloacale M. collies M. columbinasale M. columbinum M. columborale M. conjunctivae M. cricetuli M. crocodyli M. cynos M. edwardii M. equirhinis M. falconis M. faucium M. felifaucium M. felis M. fermentans M. gallinaceum M. gallinarum M. gallopavonis M. gateae M. glycophilum M. gypis M. hominis M. hyopharyngis M. hyorhinis M. hyosynoviae M. iguanae M. indiense M. iners M. lagogenitalium M. leonicaptivi M. lipofaciens M lipophilum M. maculosum M. melegridis M. moatsii M. mobile M. molare M. mustelae M. neurolyticum M. opalescens M. orale M. oxoniensis M. pullorum M. pneumoniae M. pulmonis M. salivarium M. simbae M. sp. ovine/caprine M. spermatophilum M. sphenisci M. spumans M. sturni M. sualvi M. subdolum M. synoviae M. testudineum M. timone M. turnidae M. verecundum M. zalophi

Section 6 – METHOD OF USE

However, with this kit the user can perform two types of tests depending on the demand of sensitivity and urgent response, for accurate and sensitive detection **MycoQuant™ Sensitive** protocol is recommended.
Samples should be derived from cultures, which are at app. 90 % confluence. PCR inhibiting substances may accumulate in the medium of older cultures. For a sample from an older culture, a DNA extraction and running **MycoQuant™ Sensitive** protocol is strictly recommended.

1) MycoQuant™ Fast: Detecting infection from cell culture supernatant (250 µl), fastest way of detection.

2) MycoQuant™ Sensitive: Using 20-100 ng (optimal DNA template is 50 ng in 25 µl of reaction mixture) of isolated genomic DNA, more sensitive test to detect mycoplasma infection.

MycoQuant™ Fast detection qPCR protocol

1. Gather 250 µl media above your cell culture.
2. Spin the samples for 3 min on 13 000 rpm.
3. Discard supernatant by gentle pipetting. To get rid of remaining supernatant centrifugation can be repeated for 20 sec on maximum speed (13 000 rpm).
4. Resuspend pellet (not always visible) in 10 µl **Fast Preparation buffer** by vigorous pipetting.
5. Add 12.5 µl of the **2x Reaction Mix solution** and 2.5 µl of the **Primer Mix solution** to get 25 µl final PCR reaction volume.
6. Setup your qPCR program (Dye: FAM/VIC):

	1. Hold	**2. Amplification: 40 cycles**	
Temp.	95 °C	95 °C	60 °C*
Time	120 s	15 s	30 s

* Acquire fluorescence data for both FAM and VIC channels

7. Start qPCR run.
8. Interpret data according to Section 7.

MycoQuant™ Sensitive detection qPCR protocol

1. Purify genomic DNA from cells (reagents for purification is not provided by the kit). Please be sure to remove any alcohol containing wash buffer from the preparation to avoid alcohol in the sample material, as any remaining alcohol may inhibit Taq polymerase in the PCR.
2. After quantification of DNA, dilute sample in order to have 50 ng DNA in 10 µl (10-100 ng can be used).
3. Add 12.5 µl of the **2x Reaction Mix solution** and 2.5 µl of the **Primer Mix solution** to get 25 µl final PCR reaction volume.
4. Setup your qPCR program (Dye: FAM/VIC):

	1. Hold	**2. Amplification: 40 cycles**	
Temp.	95 °C	95 °C	60 °C*
Time	120 s	15 s	30 s

* Acquire fluorescence data for both FAM and VIC channels

5. Start qPCR run.
6. Interpret data according to Section 7.

Performing PCR on the following Real-Time PCR instruments has been successfully tested: Roche, Thermo, ABI, BioRad, RotorGene, Bioneer.
Use a calibrated thermocycler for the fluorescence channel reading FAM™, TAMRA™, VIC® or equivalent.

Section 7 – INTERPRETATION OF DATA

Positive amplification in the FAM channel correspond to mycoplasma DNA in the sample. Amplification after 35 cycles should be defined as negative for mycoplasma.
Positive Control DNA should result in amplification with Ct value between 18-24 depending on the instrument.
Internal Positive Control Results
All samples should give positive amplification of the Internal Positive Control DNA PCR Assay (VIC/HEX) meaning that no PCR inhibitor was present in the reaction.

Section 8 – TROUBLESHOOTING

No Internal Positive Control signal and no target-specific signal in the wells	**PCR is inhibited**	**Repeat the assay in advised conditions diluting the sample 1:10**
		Use a purification kit adapted to your sample then repeat the assay in advised conditions
	Damaged reagents	**Repeat the assay using properly stored reagents**
		Avoid freeze/thaw cycles
	Pipetting errors	**Repeat the assay in advised conditions. Pay attention to proper pipetting. Make sure the QPCR equipment is not contaminated and correctly calibrated**
No Internal Positive Control signal, but target-specific signal in the target wells are present	**High copy number of mycoplasma DNA resulting in preferential amplification of target-specific DNA**	**Optional confirmation : repeat the assay in advised conditions diluting the sample 1:10**
No target-specific signal with the Positive control DNA (FAM), and no positive reaction with the Internal Positive Control (VIC)	**Damaged reagents**	**Repeat the assay using properly stored reagents**
		Avoid freeze/thaw cycles
No target-specific signal with the Positive control DNA (FAM), but positive reaction with the Internal Positive Control (VIC)	**Pipetting errors**	**Repeat the assay in advised conditions. Pay attention to proper pipetting of the Positive Control DNA.**
Target-specific signal (FAM) in the negative control well, and positive reaction with the Internal Positive Control (VIC)	**Contamination by samples or Positive Control DNA**	**Repeat the assay in advised conditions. If contamination persists, repeat the assay using fresh aliquots. If contamination persist, repeat the assay in advised conditions using a new kit.**

Section 9 – RELATED PRODUCTS

In case of detecting mycoplasma infection, MycoKill™ (Avidin Ltd., Cat# MK-2L) treatment is suggested, which eliminates the infection only in three weeks. MycoKill™ can be used as a routine addition in liquid media to prevent mycoplasma and related cell wall-less bacteria contamination in mammalian cell cultures. MycoKill™ exhibits no toxicity in eukaryotic cells even at higher doses.

Section 10 – Other Information

The information contained in this TDS relates only to the material(s) designated and does not relate to use(s) in combination with any other material, process(es) and/or chemical reaction(s). Avidin provides this information in good faith and is based on our present knowledge. This TDS is provided without warranty of any kind. The recipient is responsible for ensuring that, where applicable, existing laws and guidelines are observed.

Material Safety Data Sheet

Section 1 - Product and Company Information

Product Name: **MycoQuant™ Mycoplasma quantification kit**
Cat. Code: MQ-50, MQ-100, MQ-250
Company identification: Avidin Ltd., H-6726 Also kikoto sor 11
Szeged, Hungary
+36 62/202-107

Disclaimer: All Avidin products are supplied for research and laboratory use only. Not for drug, household or other uses.

Section 2 – Hazards Identification

Emergency Overview:
OSHA Hazards
Harmful by ingestion. Irritant

Other hazards which do not result in classification
Photosensitizer

Classification according to Regulation (EC) No 1272/2008 [EU-GHS/CLP] and GHS
Acute toxicity, Oral (Category 4)
Skin irritation (Category 2)
Eye irritation (Category 2)
Specific target organ toxicity - single exposure (Category 3)

Classification according to EU Directives 67/548/EEC or 1999/45/EC
Irritating to eyes, respiratory system and skin.
Harmful if swallowed.

Labeling according Regulation (EC) No 1272/2008 [CLP] and GHS
Pictogram

Signal word Warning

Hazard statement
H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H335 May cause respiratory irritation.

Precautionary statement(s)
P261 Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

According to European Directive 67/548/EEC as amended.
Hazard symbol(s)

R-phrase(s)
R22 Harmful if swallowed.
R36/37/38 Irritating to eyes, respiratory system and skin.
S-phrase(s)
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36 Wear suitable protective clothing.

Other hazards
Photosensitizer

Section 3 – Composition/Information on Ingredient

Synonyms: RT-PCR reagents
CAS number: Not available

Section 4 – First Aid Measures

General advice: Consult a physician. Show this material safety data sheet to the doctor in attendance.
After skin contact: Immediately wash skin with soap and plenty of water. Consult a physician.
After swallowing: Never give anything by mouth to an unconscious person. Rinse mouth with water provided person is conscious. Consult a physician.
After inhalation: Remove to fresh air. If not breathing give artificial respiration. Consult a physician.
After eye contact: Immediately flush eyes with plenty of water for at least 15 minutes. Consult a physician.

Section 5 – Fire Fighting Measures

Suitable extinguishing media: Water spray, carbon dioxide, dry chemical powder or appropriate foam.
Specific hazards arising from the chemical: No data available
Special Firefighting Procedures: Wear self-contained breathing apparatus for fire fighting if necessary.

Section 6 – Accidental Release Measures

Personal precautions: Wear protective equipment. Keep unprotected persons away. Avoid dust formation.
Method for Cleaning Up: Sweep up and place in closed containers for disposal. Dispose contaminated material as waste according to section 13. Ventilate area and wash spill site after material clean-up is complete.

Section 7 – Handling and Storage

Precautions for safe handling: Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.
Storage: Store at -20°C.

Section 8 – Exposure Controls / PPE

Personal protective equipment

Respiratory protection

Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection

For prolonged or repeated contact use protective gloves.

Eye protection

Safety glasses

Hygiene measures

General industrial hygiene practice.

Section 9 – Physical / Chemical Properties

Appearance
Physical state: Liquid
Color: Transparent

Safety Data
Odor: No data available
Odor threshold: No data available
pH: No data available
Melting point/freezing point: No data available
Initial boiling point and boiling range: No data available
Flash point: No data available
Evaporation rate: No data available
Flammability: No data available
Upper/lower flammability or explosive limits: No data available
Vapor pressure: No data available
Relative density: No data available
Solubility: No data available
Partition coefficient: n-octanol/water: No data available
Autoignition temperature: No data available
Decomposition temperature: No data available
Viscosity: No data available

Section 10 – Stability and Reactivity

Reactivity: No data available
Chemical stability: Stable under recommended storage conditions.
Possibility of hazardous reactions: No data available
Conditions to avoid: No data available
Incompatible materials: No data available
Hazardous decomposition products: No data available

Section 11 – Toxicological Information

Acute toxicity:
Oral LD50: No data available
Inhalation LC50: No data available
Dermal LD50: No data available
Other information on acute toxicity: No data available

Skin corrosion/irritation: No data available
Serious eye damage/irritation: No data available
Respiratory or skin sensitization: No data available
Germ cell mutagenicity: No data available
Carcinogenicity: No data available
Reproductive toxicity: No data available
Additional information: No data available

Section 12 – Ecological Information

Ecotoxicity: No data available
Persistence and degradability: No data available
Bioaccumulative potential: No data available
Mobility in soil: No data available

Section 13 – Disposal Considerations

Product: Observe all federal, state and local environmental regulations. Contact a licensed professional waste disposal service to dispose of this material. Must not be disposed of together with household garbage.
Contaminated Packaging: Dispose of as unused product.

Section 14 – Transport Information

ADR/RID: Not dangerous goods
DOT (US): Not dangerous goods
IMDG: Not dangerous goods
IATA: Not dangerous goods

Section 15 – Regulatory Information

This safety datasheet complies with the requirements of Regulation (EC) No. 1907/2006.

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture
no data available

15.2 Chemical Safety Assessment
no data available

OSHA Hazards
Harmful by ingestion. Irritant

SARA 302 Component: None of the ingredients are listed.
SARA 313 Component: None of the ingredients are listed.
SARA 311/312 Hazards: Acute Health Hazard

Section 16 – Other Information

The information contained in this MSDS relates only to the material(s) designated and does not relate to use(s) in combination with any other material, process(es) and/or chemical reaction(s). Avidin provides this information in good faith and is based on our present knowledge. This MSDS is provided without warranty of any kind. The recipient is responsible for ensuring that, where applicable, existing laws and guidelines are observed.