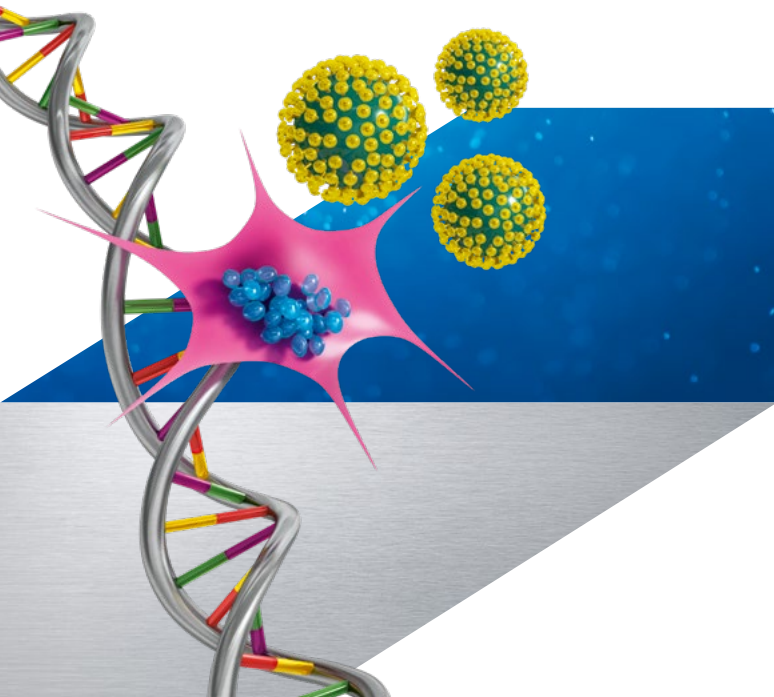


DNA Preparation for the MycoTOOL Mycoplasma Real-Time PCR Kit

Evaluation of three DNA purification methods

Roche CustomBiotech provides innovative solutions for mycoplasma testing, including the MycoTOOL Mycoplasma Real-Time PCR Kit, which is used for quality control of select biopharmaceuticals. The kit has been validated according to European Pharmacopoeia (EP) chapter 2.6.7. guidelines for validation of nucleic acid amplification techniques (NAT) for mycoplasma detection.


Roche offers multiple DNA purification methods, and evaluated two of them in comparison to the validated method for preparing DNA for testing with the MycoTOOL Mycoplasma Real-Time PCR Kit. Results show that all three methods perform comparably.



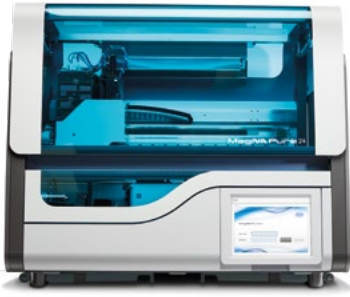
Purification Methods

Three Roche DNA purification methods were evaluated for efficiency in preparing samples for PCR analysis with the MycoTOOL Mycoplasma Real-Time PCR Kit.

AUTOMATED



MagNA Pure 96 System (Validated method)
Full validation report available under Confidential Disclosure Agreement. Summary available at http://go.roche.com/mycotool_poster

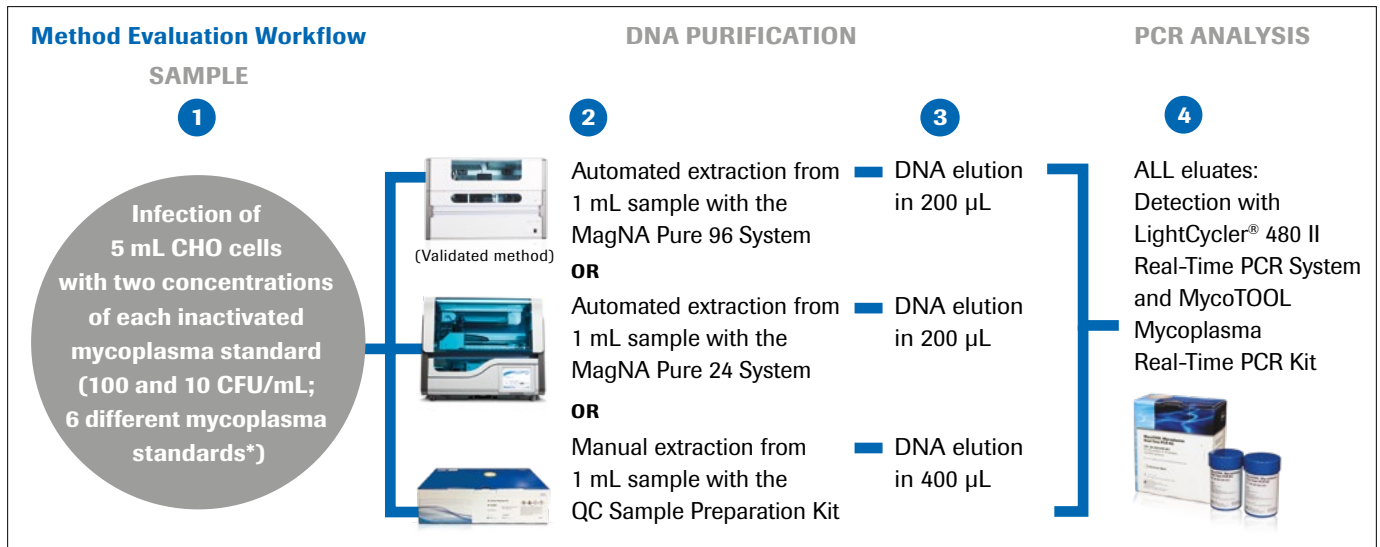


MagNA Pure 24 System

MANUAL



QC Sample Preparation Kit



*Standards recommended by EP 2.6.7. were used: *A. laidlawii*, *M. orale*, *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. pneumoniae*. Source: Mycoplasma Biosafety Service, Vienna

Notes

- CHO cells were kept frozen until use.

- DNA extraction with the MagNA Pure 96 system or the QC Sample Preparation Kit was performed according to the MycoTOOL Mycoplasma Real-Time PCR Kit Instructions for Use v9. DNA extraction with the MagNA Pure 24 System was performed according to the Instructions for Use of the Pathogen Universal Protocol.
- General acceptance criteria for the results were that all controls (positive control, recovery control, negative control) yielded the expected signal according to the MycoTOOL Mycoplasma Real-Time PCR Kit Instructions for Use. Only then was a positive result regarded as truly positive.

Results and Summary (Data on file at Roche)

Standard	Purification Method	100 CFU/mL	10 CFU/mL [‡]
A. laidlawii	MagNA Pure 96 System	Detected (CP 36.91)	Detected (CP 40.88)
	MagNA Pure 24 System	Detected (CP 36.88)	Detected (CP 39.42)
	QC Sample Preparation Kit	Detected (CP 37.07)	Detected (CP 39.69)
M. arginini	MagNA Pure 96 System	Detected (CP 38.36)	Detected (CP 40.15)
	MagNA Pure 24 System	Detected (CP 38.71)	Detected (CP 40.43)
	QC Sample Preparation Kit	Detected (CP 38.23)	Detected (CP 41.63)
M. fermentans	MagNA Pure 96 System	Detected (CP 37.95)	Detected (CP 41.16)
	MagNA Pure 24 System	Detected (CP 39.78)	Detected (CP 43.52)
	QC Sample Preparation Kit	Detected (CP 38.88)	Detected (CP 45.70)
M. pneumoniae	MagNA Pure 96 System	Detected (CP 37.02)	Detected (CP 40.35)
	MagNA Pure 24 System	Detected (CP 34.78)	Detected (CP 42.83)
	QC Sample Preparation Kit	Detected (CP 36.85)	Detected (CP 42.83)
M. hyorhinis	MagNA Pure 96 System [†]	Detected (CP 37.24)	Detected (CP 41.32)
	MagNA Pure 24 System	Detected (CP 36.56)	Detected (CP 39.92)
	QC Sample Preparation Kit	Detected (CP 37.32)	Detected (CP 40.62)
M. orale	MagNA Pure 96 System	Detected (CP 35.67)	Detected (CP 39.47)
	MagNA Pure 24 System	Detected (CP 36.83)	Detected (CP 43.97)
	QC Sample Preparation Kit	Detected (CP 36.14)	Detected (CP 41.48)

CP values shown in the table refer to the average of four technical replicates.

[†] Negative Control showed a positive signal due to end-user pipetting error.

[‡] According to the MycoTOOL Mycoplasma Real-Time PCR Kit Instructions for Use, a biological sample is regarded as positive if at least one of four technical replicates yields a positive signal. Thus, samples with a CFU concentration close to the limit of detection (10 CFU/mL) show a higher variance in CP values between technical replicates. If one of the four technical replicates returned a negative signal, CP values were averaged based on three instead of four replicates.

Contact your CustomBiotech representative for further information or visit custombiotech.roche.com/mycotool.

Europe, Middle East, Africa, Latin America
mannheim.custombiotech@roche.com

United States / Canada
custombiotech.ussales@roche.com

Asia Pacific
apac.custombiotech@roche.com

Japan
japan.custombiotech@roche.com

The three purification methods perform comparably. All six species were detected in both concentrations. **The three methods can be recommended as a DNA isolation and purification method for the MycoTOOL Mycoplasma Real-Time PCR Kit.** (Product-specific validation required.)

Crossing point (CP) analysis shows that **DNA purification efficiency varies slightly** across the three tested methods.

MycoTOOL Mycoplasma Real-Time PCR and QC Sample Preparation kits are for use in quality control / manufacturing process only.

All MagNA Pure 24 and MagNA Pure 96 kits, consumables, and accessories are for in vitro diagnostic use unless otherwise noted.

The LightCycler® 480 system is for life science research use only. Not for use in diagnostic procedures.

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Published by
Roche Diagnostics GmbH
Sandhofer Straße 116
68305 Mannheim
Germany

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