HumqPCR-realtime™ Microspores gypseum

BioinGentech
HumqPCR - Real Time™

Microspores gypseum - Real time

50 / 100 /150 tests (Ready to use kit)

HumqPCR-realtime™ Microspores gypseum Real Time PCR Kit is a screening assay for the detection of Microspores gypseum.

Principles of the test

The Microspores gypseum primer and probe mix are provided and this can be detected through the yellow channel.
During PCR amplification, forwar and reverse primers hybridize to the Microspores gypseum. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5-dye Chods ZX™ and 3-quencher kurü Zy™. Internal control consists of a DNA probe labeled with a 5-dye kellú ZZ™ and a 3-quencher kurü Zy™.
During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of realtime PCR platforms.

Principle and use:

This amplification kit has been manufactured by Bioingentech Ltd. Chile to detect Microspores gypseum in real time PCR. This is a posibility absolute quantification or qualitative assay.
Real time PCR is based on fluorogenic dyes. Up to 36 Ct should be taken positive. Value between 36-40 Ct should be taken as marginal positive.
This kit needs DNA which can be isolated from blood, serum, faeces, respiratory fluid, digestive system, tissue and others.
See (PU-A001, PU-A002 & PU-A003) included in advanced formats.

Kit Components

Pulse-spin each tube in a centrifuge before opening.
Completely thaw the components of the kit prior to use.
Homogenize the solutions for 5 seconds prior to pipetting.

Reactions	50 test	100 test	150 test
HumqPCR-realtime™ Microspores gypseum	(1 vial)	(1 vial)	(1 vial)
HumqPCR-realtime™ Internal Control	(1 vial)	(1 vial)	(1 vial)
PCR Grade water	(1 vial)	(1 vial)	(1 vial)
Microspores gypseum Positive control	(1 vial)	(1 vial)	(1 vial)
Microspores gypseum Negative control	(1 vial)	(1 vial)	(1 vial)

Instrument Compatibility in:

* Ask us for information.

* ABI 7300	* LightCycler 2.0
* ABI 7500FAST	* LightCycler 480
* ABI 7900	* Mastercycler® ep realplex
* AB Step One	* Mx3000P QPCR System
* AB Step One Plus	* Mx3005P QPCR System
* Agilent Mx3005P	* RotorGene 3000
* CFX96 & CFX384	* RotorGene 6000
* ExiCyclerTM 96	* RorotGeneQ
* iQ5 & MyiQ Cycler	* SLAN® Real-Time PCR
* Illumina Eco	* Smartcycles II
* LightCycler Nano	* Applied 7300 and 7500

Procedure:

Please read through the entire procedure before starting.

STEP 1

Preparation of Microspores gypseum Mixture

1) Prepare the reaction mixture for sample, positive control, negative control, and internal control by combining the reagents as shown in the table 1. The final reaction volume should be 13.5 µL

Notes:

- Run a positive control, a negative control, and an internal control for each 12 samples.

- The mineral oil is necessary, only when using a thermalcycler that employs a top heating method.

Table 1. Reaction components for PCR

	Sample	Positive control	Negative control	Internal control
HumqPCR-realtime™ Microspores gypseum	5.5µL	5.5µL	5.5µL	
HumqPCR-realtime™ Internal Control				5.5 µL
PCR Grade water	6µL	6µL	6µL	6µL
DNA isolated from the sample	2µL			2µL
Microspores gypseum Positive control		2µL		
Microspores gypseum Negative control			2µL	
Total Volumen	13.5µL	13.5µL	13.5µL	13.5µL

Visual explanation:

Sample:

2µl DNA isolated from the sample
6µl PCR Grade water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

Positive Control:

2µl Microspores gypseum Positive control
6µl PCR Grade water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

Negative Control:

2µl Microspores gypseum Negative control
6µl PCR Grade water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

Internal Control:

2µl DNA isolated from the sample
6µl PCR Grade water
5.5µl HumqPCR-realtime™ Internal Control

Total: 13,5 µL

Observation:
homogenize solution in each tube during 10 seconds.

Place the tubes in a Instrument and perform amplification according to the program outlined in Table 2.

Table 2. Real Time cycling parameters

PCR cycle		Temp.	Time
x 1 cycles	Initial Denaturation	94ºC	2 min.
x 45 cycles	Denaturation	95°C	15 seconds
	Annealing	60°C	60 seconds

Step 3

Once the program will be finished one can see the graphics. The negative control should run along with the bottom and positive control must give a curve in the software graphics. Use your software to analyse the results.

Interpretation of the test

1) Qualitative analysis:

Ct (Threshold cycle) value of each sample can be read as follows.

Ct value result

> 40 negative	≤ 40 positive

2) Quantitative analysis:

Table 3. Preparation of standard curve dilution series. Microspores gypseum positive control:

Average Positive Control Concentration	
Microspores gypseum	See quality control

Standar curve	Preparation series a fresh dilution	Concentration	Copy Number
Tube N°1:	2uL Microspores gypseum Positive Control (0,3 ng/µl) + 18uL PCR Grade Water	See quality control	See quality control
Tube N°2:	2uL Tube N°1 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°3:	2uL Tube N°2 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°4:	2uL Tube N°3 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°5:	2uL Tube N°4 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°6:	2uL Tube N°5 + 18uL PCR Grade Water	See quality control	See quality control
Tube N°7:	2uL Tube N°6 + 18uL PCR Grade Water	See quality control	See quality control

Homogenize tube.

*** We will send a Quality Control report for each purchase**
**** If you want to obtain 13 DNA copies you must include a new dilution tube (Tube N° 8)**

Table 4. Reaction components for Real Time PCR standar curve.

	Tube A	Tube B	Tube C	Tube D	Tube E	Tube F	Tube G
HumqPCR Microspores gypseum	5.5 µl	5.5 µl	5.5 µl	5.5 µl	5.5 µl	5.5 µl	5.5 µl
PCR Grade Water	6.0 µl	6.0 µl	6.0 µl	6.0 µl	6.0 µl	6.0 µl	6.0 µl
Tube N°1	2.0 µl						
Tube N°2		2.0 µl					
Tube N°3			2.0 µl				
Tube N°4				2.0 µl			
Tube N°5					2.0 µl		
Tube N°6						2.0 µl	
Tube N°7							2.0 µl

2.1.-Assess the Ct value when amplification curve of Standard tube 1, 2, 3, 4, 5, 6 passes the threshold line. However four tubes is sufficient for standard curve. (tube1-tube4).

2.2.- Calculate quantitative value to compare with Ct value of unknown samples and curve of Standard tube 1, 2, 3, 4, 5, 6.

3) Test validation:

3.1.- Each Ct value standard should be as follows.
Standard 1 > Standard 2 > Standard 3 > Standard 4> Standard 5 > Standard 6.

3.2.- R-value of standard curve should be 0.900~0.999.

3.3.- The standard result should be all negative.

Visual explanation:

Tube A

2.0µl Tube N°1 (Positive Control)
6.0µl PCR Grade Water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

Tube B

2.0µl Tube N°2
6.0µl PCR Grade Water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

Tube C

2.0µl Tube N°3
6.0µl PCR Grade Water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

Tube D

2.0µl Tube N°4
6.0µl PCR Grade Water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

Tube E

2.0µl Tube N°5
6.0µl PCR Grade Water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

Tube F

2.0µl Tube N°6
6.0µl PCR Grade Water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

Tube G

2.0µl Tube N°7
6.0µl PCR Grade Water
5.5µl HumqPCR-realtime™ Microspores gypseum

Total: 13,5 µL

FAQ:

1.-Positive control:

The positive control assay uses a Chods Zx™ dye and should be detected through the Yellow channel of your real time PCR instrument.
For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of Microspores gypseum copy number/CT value. Alternatively the positive control can be used at a single dilution Microspores gypseum on where full quantitative analysis of the sample is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primer and probes for detecting the target Microspores gypseum gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. sealing all other samples and negative controls before pipetting the positive control into the positive control well tube.

2.-Internal Control:

The internal control assay uses a kellú ZZ™ dye and should be detected through the Green channel of your real time PCR instrument. A positive result through the Green channel therefore indicates that PCR conditions are suitable for detection of the target pathogen gene. If a negative result is obtained through the Green channel the results should be ignored and the test repeated.

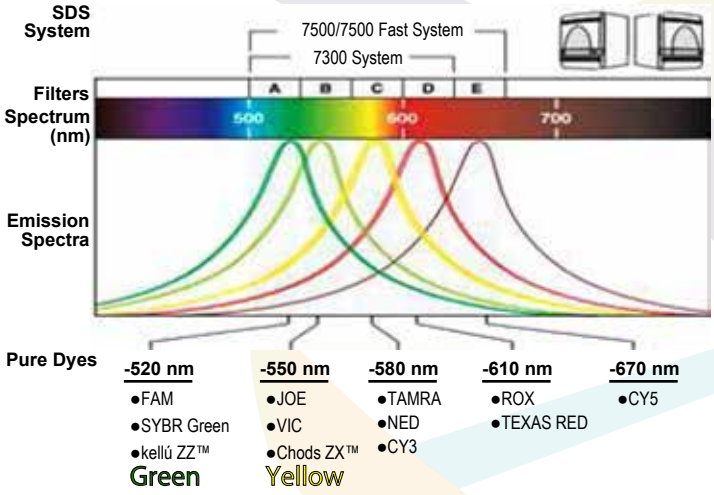
3.-Negative control:

To confirm absence of contamination a negative control reaction should be included every time the kit is used. In this instance the PCR grade water should be used in place of template. A negative result indicates that the reagents have not become contaminated. If a positive result (CT value less than 37) is obtained the results should be ignored and the test repeated.

You must use quencher and reporter dye to setup your software (see table 2) and run the following channel:

Table 5. Report Dye channel selection chart

Channel	Source	Detector	Dyes
Green	470 (Nm)	520 (Nm)	FAM, Sybr green1, Fluorescein, Eva green, Alerxa flour 488, kellú ZZ™
Yellow	530 (Nm)	550 (Nm)	Joe, Vic, Hex, Tet, Cal Fluorgold 540, YaKima Yellow, Chods ZX™
Orange	585 (Nm)	610 (Nm)	Rox, Cal Fluor Red 610, Cy3.5, Texas Red, Alexa Fluor 568
Red	625 (Nm)	660 (Nm)	Cy5, Quasar 670, Lightcycler, Red 640, Alexa Fluor 633.
Crimson	680 (Nm)	710 (Nm)	Quasar 705, Lightcycler Red 705, Alexa Fluor 680



Similarity

Similarity of our fluorophores with HEX and FAM.

•kellú ZZ™	FAM
•Chods ZX™	HEX

Temperature

All our reagents are made through protein engineering and are stable at room temperature, the label temperature is just a recommendation after the product is open.

Store temperature	The label temperature
Shipping temperature	At room temperature

Table 6. Interpretation of Results

Target	Internal Control	Negative Control	Positive Control	Interpretation
+ive	+ive	-ive	+ive	+ive
+ive	-ive	-ive	+ive	+ive
+ive	+ive	+ive	+ive	*
+ive	-ive	+ive	+ive	*
-ive	+ive	-ive or +ive	+ive	-ive
-ive	-ive	-ive or +ive	-ive	Experiment fail
-ive	+ive	-ive or +ive	-ive	Experiment fail

Table 7. Products

Products		Code
Bioingentech - Genomic DNA Purification Kit	50 test	PU-A001
Bioingentech - Genomic DNA Purification Kit	100 test	PU-A002
Bioingentech - Genomic DNA Purification Kit	150 test	PU-A003
HumqPCR-realtime™ Microspores gypseum Real Time 50 tests (Ready to use kit) / Cat. No: RTq-H701-50D		
HumqPCR-realtime™ Microspores gypseum Real Time 100 tests (Ready to use kit) / Cat. No: RTq-H701-100D		
HumqPCR-realtime™ Microspores gypseum Real Time 150 tests (Ready to use kit) / Cat. No: RTq-H701-150D		