

Vaginosis Test Panel (100) reactions

Not intended for human or animal, diagnostic or therapeutic uses. **Storage (-20^o C)**

For research use only

Kit (VAG – 0240) Contains:

- VAG-MPCR Primers
- VAG-Sequencing Primers
- Enhancer
- Dnase free water

Suggested Accessories:

- Big Dye Terminator (Life Technologies Version 1.1 Cat no. 4337450)
- Qiagen Multiplex master mix Cat No 206152
- Ampure-Xp (Beckman Coulter USA A 63881)
- CleanSeq (Beckman Coulter USA Cat No. 000121)

Usage: Reagents in this kit can be used to simultaneously generate nucleotide sequences from *C.albicans*, *G.vaginalis*, *T.vaginalis*, *Herpes simplex 1* and *Herpes simplex 2*. This kit provides enough reagents to perform 100 tests following the protocol outlined below:

Protocol:

MPCR Reaction	
REAGENT	Vol/Rx
Qiagen Buffer	25 ul
MPCR primers	10 ul
DNA Extract	10 ul
Dnase free water	5 ul
Rx Vol	50 ul

Thermocycling profile	
Pre cycle 95 ^o C	5 min
95 ^o C	30 Sec
56.0 ^o C	90 Sec
72 ^o C	30 Sec
No. Cycles	35
Hold time	68 ^o C/10 min

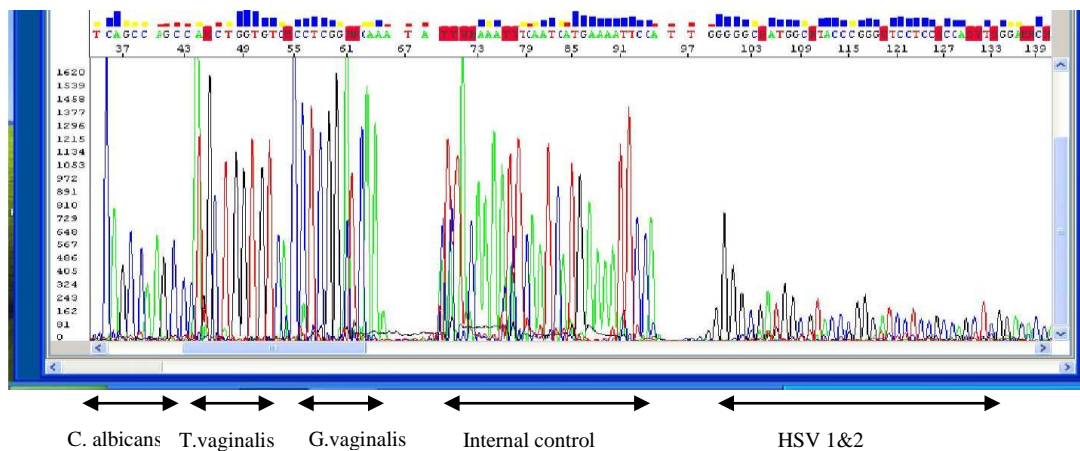
A. MPCR Clean up:

- Add 5 ul of the enhancer; incubate at 37^o C for 60 min, heat at 95^o C for 3 min, 4^o C/hold.
- Purify MPCR reactions using Ampure according to the instructions provided (Beckman Coulter, USA).

Sequencing Reaction	
REAGENT	Vol/Rx
Big Dye	1.0 ul
5X Sequencing Buffer	3.5 ul
Sequencing Primers	5.0 ul
Template	8.0 ul
D.water	2.5 ul
Rx Vol	20 ul

Thermocycling profile	
96° C	15 Sec
50° C	8 Sec
60° C	2.5 Min
No. Cycles	25
Hold temp	4° C

- A. Cycle Sequencing Excess Dye Terminator Removal:** Purify cycle sequencing reactions using Cleanseq according to the instructions provided (Beckman Coulter, USA).
- B. Analysis:** Sequence the sample using capillary electrophoresis. Follow the manufacturer's instructions for the preparation of the sample. Using Sequencing Analysis Software Version 5.2 (Life Technologies, USA) and an ABI Prism™ 3130 Genetic Analyzer (Life Technologies, USA), the expected electropherogram is:



Organism	Read Sequence
C.albicans	CAGCCAAGCCC
G.vaginalis	CCTCGGATCAA
T.vaginalis	ATGCTGGTGTCA
Herpes Simplex 1	GGGGCGATGGCGTATGCGGGATCCTCGG
Herpes Simplex 2	GGGGCGATGGCGTACCCGGGATCCTCCG
IC-Kras	TTACAAAATTC AATCATGAAAATTCCA