molecular designs

DOC-VAG384-INSERT-B

Vaginal Microbiota *Simplicity Panel™* 384-Well

Product Specification Sheet

RUO Product*

Product Description:

The Vaginal Microbiota *Simplicity Panel™* is an *in-vitro* multiplex real-time polymerase chain reaction (RT-PCR) assay for the qualitative identification of nucleic acids from organisms frequently found in the vagina. This method is highly accurate, analytically sensitive, and is used to identify organisms by amplifying and detecting genetic material of pathogens in samples. The panel will aid in the research of causative agents of vaginal infections and their prevalence.

The target organisms included in the panel are as follows: *Atopobium vaginae, Bacteroides fragilis, Bacterial Vaginosis Associated Bacterium-2, Candida albicans, Candida dubliniensis, Candida glabrata, Candida krusei, Candida lusitaniae, Candida parapsiliosis, Candida tropicalis, Chlamydia trachomatis, Escherichia coli, Enterococcus spp., Gardenella vaginalis, Haemophilus ducreyi*, Herpes Simplex virus-1, Herpes Simplex virus-2, *Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners, Lactobacillus jensenii, Megasphaera Type 1, Megasphaera Type 2, Mobiluncus curtisii, Mobiluncus mulieris, Mycoplasma genitalium, Mycoplasma hominis, Neisseria gonorrhoeae, Prevotella bivia, Staphylococcus aureus, Streptococcus agalactiae, Treponema pallidum, Trichomonas vaginalis,* and *Ureaplasma urealyticum.*

Product Information	
Vaginal Microbiota Panel *Simplicity Panel™* (384-Well Plate)	
Part Number	P-VAG384-001-A P-VAG384-002-A
Number of Panels	12
Positive Control	*Gardnerella vaginalis*
Storage Temperature	-25°C to -15°C

Product Specifications	
QC Test	qPCR Cycle Threshold Percent CV
Specification	≤ 2.5

QC Results	
Positive	meets specification
Negative	meets specification
Targets	meets specification

Disclaimer - Use of PCR and Patent

This product is for basic PCR and is outside of any valid US patents assigned to Hoffman La-Roche.

ISO Certification

This product was manufactured in a facility whose Quality Management System is certified as being in conformity with ISO 13485:2016 by Intertek.

* Limitations of Use

For Research Use Only. Not for use in diagnostic procedures.

Product Guarantee

This kit is proven in PCR and generates reliable, repeatable and high-performance results. Please contact Molecular Designs for technical assistance. If not completely satisfied, our team will help you identify and address the issue and replace the assays as needed.

Usage Information

Reagent Storage and Use Guidelines

1. Store all reagents at -25°C to -15°C.
2. Do not freeze-thaw plates more than 3 times.

The Following is Included in the Kit:

1. 384-well PCR plate pre-loaded with the Vaginal Microbiota *Simplicity Panel*™ assays and positive control. Negative control assay is plated but negative control is user supplied.

The Following is Supplied by the User: Materials

1. Extracted Sample(s)
2. qPCR optical film
3. Sealer for optical film
4. Negative Control

Equipment

1. Manual defrost -20°C freezer
2. Laminar Flow or PCR Dead Air Box for general plate setup. Do not use Laminar Flow for infectious samples
3. Pipette and appropriate filtered pipette tips
4. Plate Vortex [recommend Vortex Genie 2 (Model G560) with the 3-inch platform and rubber cover]
5. Plate centrifuge

Instrumentation

1. CFX384 Touch Real-Time PCR Detection System (or equivalent)

General Guidelines and Safety Precautions

1. As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.
   a) Do not pipette by mouth.
   b) Do not eat, drink, or smoke in designated work areas.
   c) Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples to prevent contamination. Avoid contaminating gloves when handling samples and controls.
   d) Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
   e) Thoroughly clean and disinfect all laboratory work surfaces.
   NOTE: Do not use sodium hypochlorite solution (bleach) to clean up a spill or to disinfect a plate before disposal as it can react with the common extraction reagents and generate toxic byproducts.
   If spills occur, follow internal procedures to immediately clean and decontaminate the surface of instrument.
2. A laminar flow or PCR Dead Air Box is recommended to reduce contamination probability.
3. The use of filtered, sterile and nuclease-free pipette tips is recommended.
4. False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Manufacturer of Record: Molecular Designs, LLC
2 Perimeter Park, Birmingham, AL 35243
www.moleculardesigns.com

Usage Information

Reaction Plate Setup

1. Remove a reaction plate from the -20°C manual defrost freezer.
2. Use the plate within 1 hour of thawing, keep sealed and store refrigerated at 4°C if not using immediately.
3. Spin down the plate for 30 seconds in a plate centrifuge.
4. Carefully remove the foil seal from the plate.
5. Add 4.0 µL of the sample being tested to each of the target wells.
   NOTE: 384 well plates have 16 rows (labeled A:P) and 24 columns (labeled 1:24). When plating samples, alternate rows are pipetted to accommodate the use of a multichannel pipette. Refer to page 5 of this document for the detailed well/ panel layout.
6. Do not add any additional liquid to the Positive Control. All components have been added to these wells.
7. Add 4.0 µL of negative control (user provided) to the Negative Control well.
8. Seal the PCR plate using optical qPCR film.
9. Optional: vortex the plate, at least 5 seconds per plate quadrant.
10. Optional: spin down the plate in a plate centrifuge.

Procedural Notes

1. Do not reuse consumables. They are for one-time use only.
2. Always use caution when transferring specimens from a primary collection tube to a secondary tube.
3. Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.
4. Always use a new pipette tip for each specimen.
5. For testing of previously frozen sample, ensure samples are equilibrated to room temperature and well mixed prior to use.

Target Layout per panel (384-well plate, 12 panels per plate)

See Page 5 for the complete layout of a 384-well plate.

A. vaginae	T. vaginalis	L. jensenii (VIC)	M. curtisii
C. trachomatis	C. albicans/ C. parapsilosis (CFO)	BVAB-2/ Meg Type 2 (VIC)	S. aureus
G. vaginalis	C. krusei	Meg Type 1	S. agalactiae
H. ducreyi	C. lusitaniae	B. fraglis	U. urealyticum
HSV-1	C. glabrata/ C. dubliniensis (CFO)	Enterococcus spp (CFO)	Endogenous Control
HSV-2	C. tropicalis/ P. bivia (CFO)	E. coli (CFO)	Empty
N. gonorrhoeae	L. crispatus/ L. gasseri (VIC)	M. genitalium (VIC)	Positive Control
T.pallidum	L.iners / M. mulieris (VIC)	M. hominis (CFO)	Negative Control

If not noted, the fluorophore is FAM and the second channel is noted in parentheses signifying a secondary target in the primer and probe mixture which will be detected on the qPCR instrument. CFO is equivalent to CAL Fluor Orange 560.

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Usage Information

Real-Time PCR Detection System qPCR Run Setup

1. Open the specified run template and fill in the sample name fields with unique sample IDs corresponding to the samples being processed.
2. **NOTE**: This step can also be done prior to reaction plate setup if sample IDs have already been specified.
3. Place the reaction plate into the instrument in the appropriate orientation (A1 in the upper left corner), close the instrument lid and initiate the run.

Thermocycling Protocol

1. Reverse Transcription
   a) 5 minutes at 50°C
2. Denaturation
   a) 3 minutes at 95°C
3. Annealing and Extension
   40 cycles consisting of:
   a) 5 seconds at 95°C
   b) 30 seconds at 60°C, with fluorescence acquisition during this step

Amplification Interpretation and Troubleshooting

1. The laboratory should establish cycle threshold (CT) cutoffs as appropriate for their sample workflow and procedures. It is recommended that CT cutoffs are determined during the validation of the test.
2. The laboratory should evaluate the curve shape when considering whether a sample with a given CT should be considered positive:
   a) Plate sealing issues can lead to jagged curve shapes or rising/decreasing baselines that lead to inaccurate data (erroneous CT value).
   b) Inappropriate mixing or centrifuging can lead to inaccurate data.
3. If user suspects contamination, it is recommended to clean and disinfect the laboratory area and re-test to ensure proper results.
4. Any failure of the positive or negative control should require a repeat run. If the control failure continues, it is recommended to have the qPCR instrument and the sample extraction workflow evaluated to ensure they are functioning properly.

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Vaginitis Microbiota *Simplicity Panel*™ 384-Well – 12 Panels

	Patient 1 (white) and 2 (grey)				Patient 3 (dark grey) and 4 (black)				Patient 5 (white) and 6 (grey)				Patient 7 (dark grey) and 8 (black)				Patient 9 (white) and 10 (grey)				Patient 11 (dark grey) and 12 (black)			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt
B	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt	A. vag	T. vag	L. jen (VIC)	M. curt
C	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur
D	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur	C. tracho	C. alb / C. para (CFO)	BVAB-2 / Meg Type 2 (VIC)	S. aur
E	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga
F	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga	G. vag	C. kru	Meg Type 1	S. aga
G	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea
H	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea	H. duc	C. lus	B. frag	U. urea
I	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control
J	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control	HSV-1	C. glab / C. dub (CFO)	Entero spp (CFO)	Endo Control
K	HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)	
L	HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)		HSV-2	C. trop / P. biv (CFO)	E. coli (CFO)	
M	N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)	
N	N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)		N. gono	L. cris / L. gas (VIC)	M. gen (VIC)	
O	T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)	Positive Control
P	T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)		T.pal	L. ine / M. mulieris (VIC)	M. hom (CFO)	Negative Control

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