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For research purposes only

Androflor® REAL-TIME PCR Detection Kit

Androflor® Screen REAL-TIME PCR Detection Kit

General information

Intended use:
The DNA-Technology Androflor® REAL-TIME PCR Detection Kit and Androflor® Screen REAL-TIME PCR Detection Kit are intended for the study of men's urogenital tract microbiocenosis by method of Real-Time PCR.
Depending on the number of identified microorganisms Androflor® REAL-TIME PCR Detection Kit is available in the following formats:
- Androflor®;
- Androflor® Screen.

Androflor® Screen is a shortform of Androflor®.

Method:
Real-time PCR; qualitative multiplex analysis.

Samples:
Epithelial cells scrapes from the balanus, urethra; urina; prostatic fluid; ejaculate; biopsy samples from prostatic tissues.

DNA extraction:
The DNA-Technology PREP-NA PLUS and PREP-GS PLUS extraction kits are recommended.

Features:
Simultaneous detection of several DNA-targets in one tube (multiplex).
PCR-Mix contains internal control (IC) for evaluation of PCR quality.
Defined tubes contain ROX dye label – "Marker". It tags the tube/strip orientation.
We also recommend including in assay the negative control ("C-") which is not supplied but very helpful for contamination control purposes. Use deionized water or sterile buffered saline instead of sample, starting from extraction step.

Devices:
The automatic analysis for Androflor® and Androflor® Screen REAL-TIME PCR Detection Kits is available on "DNA-Technology" made DTlite[1], DTprime[2] and DT-96 REAL-TIME Thermal Cyclers; software version is not lower than 7.7.5.42; the current version of the software is available for download at http://www.dna-technology.ru/eng/support/.

⚠ Please enquire DNA-Technology company's representative about compatibility of third-party Real-time instruments.

Overall time needed to perform the analysis (not including sample preparation procedure):
2.5 hours.

The number of tests:
12[3]/24[4]

[1] - supported by 4S1, 4S2, 5S1, 5S2, 6S1, 6S2 instruments
[2] - supported by 4M1, 4M3, 4M6, 5M1, 5M3, 5M6, 6M1, 6M3, 6M6 instruments
[3] - Androflor® format
[4] - Androflor® Screen format

Kit contents:

Format	Androflor®		Androflor® Screen	
Reagent	The number of tubes	Quantity	The number of tubes	Quantity
Paraffin sealed PCR-mix	Strip № 1 12 8-tubes strips	20 µL in each tube	24 8-tube strips	20 µL in each tube
	Strip № 2 12 8-tubes strips	20 µL in each tube		
Taq-polymerase solution	4 tubes	500 µL in each tube	4 tubes	500 µL in each tube
Mineral oil	4 tubes	1.0 mL in each tube	4 tubes	1.0 mL in each tube
Positive control sample	1 tube	160 µL	1 tube	160 µL
Cap strips	24 8-cap strips			

Strip content, colour codes and detection channels

Androflor® format

№ of tube	Detection channel				Color of the buffer/ paraffin
	Fam	Hex	Rox	Cy5	
Strip № 1					
1	Total bacterial mass (TBM)	IC	Marker	-	Blue / White
2	*Lactobacillus spp.*	IC	*Gardnerella vaginalis*	-	
3	*Staphylococcus spp.*	IC	*Streptococcus spp.*	-	Colorless / White
4	*Megasphaera spp. / Veillonella spp. / Dialister spp.*	IC	-	-	
5	*Sneathia spp. / Leptotrichia spp. / Fusobacterium spp.*	IC	-	-	
6	*Ureaplasma urealyticum*	IC	*Ureaplasma parvum*	*Corynebacterium spp.*	
7	*Mycoplasma hominis*	IC	*Mycoplasma genitalium*	-	
8	*Bacteroides spp. / Porphyromonas spp. / Prevotella spp.*	IC	*Atopobium cluster**	-	
Strip № 2					
1	*Anaerococcus spp.*	IC	-	-	Blue / Blue
2	*Peptostreptococcus spp. / Parvimonas spp.*	IC	Marker	-	
3	*Eubacterium spp.*	IC	-	-	Colorless / Blue
4	*Haemophilus spp.*	IC	-	-	
5	*Pseudomonas aeruginosa/Ralstonia spp./ Burkholderia spp.*	IC	-	-	
6	*Enterobacteriaceae spp. / Enterococcus spp.*	IC	-	-	
7	*Candida spp.*	IC	-	Human genomic DNA	
8	*Trichomonas vaginalis*	IC	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*	

* - *Atopobium cluster* includes: *Atopobium spp., Olsenella spp., Collinsella spp.*

Androflor® Screen format

№ of tube	Detection channel				Color of the buffer/ paraffin
	Fam	Hex	Rox	Cy5	
1	Total bacterial mass (TBM)	IC	Маркер	-	Blue / White
2	*Lactobacillus spp.*	IC	*Gardnerella vaginalis*	-	
3	*Staphylococcus spp.*	IC	*Streptococcus spp.*	-	Colorless / White
4	*Ureaplasma urealyticum*	IC	*Ureaplasma parvum*	*Corynebacterium spp.*	
5	*Mycoplasma hominis*	IC	*Mycoplasma genitalium*	-	
6	*Enterobacteriaceae spp./ Enterococcus spp.*	IC	-	-	
7	*Candida spp.*	IC	-	Human genomic DNA	
8	*Trichomonas vaginalis*	IC	*Neisseria gonorrhoeae*	*Chlamydia trachomatis*	

Storage and handling requirements

The kit must be stored between 2 °C and 8 °C and out of light during the storage period. Excessive temperature and light can be detrimental to product performance.

Shelf life – 12 months from the date of production.

Procedure

1 PCR amplification

1.1 Mark the required number of strips with paraffin sealed PCR-Mix for each test sample, negative control ("C-") and positive control ("C+").

⚠ Androflor® format - two strips with paraffin-sealed PCR-mix (strip № 1 and strip № 2) are used for one sample analysis, including "C-" and "C+".
Androflor® Screen format – one strip with paraffin-sealed PCR-mix is used for one sample analysis, including "C-" and "C+".

Example. An example of strips marking for testing of 2 samples see in the Table 1.

Table 1 Marking of strips for PCR

Format	Androflor®	Androflor® Screen
Sample 1	Strip № 1 Strip № 2	Strip with paraffin-sealed PCR mix
Sample 2	Strip № 1 Strip № 2	Strip with paraffin-sealed PCR mix
«C+»	Strip № 1 Strip № 2	Strip with paraffin-sealed PCR mix
«C-»	Strip № 1 Strip № 2	Strip with paraffin-sealed PCR mix

1.2 Vortex the Taq-polymerase solution thoroughly (3-5 sec), then spin briefly (1-3 sec).
1.3 Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.
1.4 Add one drop (~20 µL) of mineral oil into each tube. Close strips tightly.
1.5 Add 5.0 µL of the DNA sample into each tube of a strip (or strips) assigned to test samples. Open the corresponding strip (or strips), add DNA sample, then close the strip (or strips) before proceeding to the next DNA sample to prevent contamination. Use filter tips. Do not add DNA into the "C-", "C+" strips.
1.6 Add 5.0 µL of the "C-" which passed whole DNA extraction procedures into tubes of corresponding strip. Add 5.0 µL of the "C+" into tubes of corresponding strip. Avoid paraffin layer break.
1.7 Spin strips briefly at 1000 RPM for 3-5 sec.
1.8 Set the strips to Real-time PCR instrument.
1.9 Launch the RealTime_PCR application in Device handling mode. Upload Androflor.ini file before the first run. In subsequent runs add test "Androflor". Specify the number and identificator of samples. Define position of tubes in software interface according to position they were set in the thermoblock (see 1.8). Run PCR.

2 The PCR and post-PCR analysis are operated by software and held in automatic mode.

Contact our customer service department regarding issues of quality Androflor® and Androflor® Screen REAL-TIME PCR Detection Kits:
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