



IS-pro Microbiota

For Research Use Only

Test Instructions

Amplification DNA assay for the relative quantitative in vitro detection of the bacterial phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Bacteroidetes* and *Proteobacteria*.

Catalogue Number: Research 14000, 24 tests

Store at -20°C upon receipt

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1. Intended use

IS-pro Microbiota kit is intended to be used for detection and identification of bacteria for research use only. Bacteria can be relatively quantified, based on their proportion of the total bacterial population within a specimen. Total peak height of a sample is correlated to absolute quantification. The intended user will be a specialized molecular laboratory. The test will be carried out by trained laboratory personnel. No special training will be required for professional laboratories performing semi-quantitative PCR.

2. Summary and explanation of the test

This IS-pro Microbiota kit is based on IS-pro™. IS-pro™ is a bacterial profiling technique based on species-specific length polymorphisms of the IS region and phylum-specific sequence polymorphisms of 16S rDNA. IS-pro™ consists of two multiplex PCRs. The first PCR is specific for *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia* and *Bacteroidetes*, the second PCR is specific for *Proteobacteria*. A marker is added to the IS-pro™ eMix as a length reference to determine lengths of the PCR amplicons.

3. Principle of the test procedure

IS-pro™ is a bacterial profiling technique based on species-specific length polymorphisms of the IS region and phylum-specific sequence polymorphisms of 16S rDNA. IS-pro™ consists of two multiplex PCRs. The first PCR reaction sorts species into the groups *Firmicutes / Actinobacteria / Fusobacteria / Verrucomicrobia* (FAFV) or *Bacteroidetes*, based on forward primer label, the second PCR is specific for *Proteobacteria*.

The IS region is amplified with fluorescently labeled phylum specific primers targeting the 16S rDNA and unlabeled primers targeting the 23S rDNA. The IS-pro™ reaction yields peak profiles that provide two levels of information: color of fragments sorts species into phyla and fragment lengths can be used to further identify bacteria to the genus, species, or subspecies level.

4. Reagents

PRECAUTIONS



**CAUTION: Handle patient samples as Biohazardous material.
Handle samples as if capable of transmitting an infectious agent.**

All clinical samples should be regarded as infectious. These samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious specimen in the Centre for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories," 1984.

Use sterile, DNase-RNase free, aerosol resistant pipette tips.

A uni-directional workflow must be adhered to in the laboratory with different rooms for sample preparation, pre-amplification area and post-amplification.

Protect kit contents or generated PCR product from direct sunlight.

Wear protective gloves.

For handling IS-pro™ eMix (Research 14007) work in a well-ventilated area, wear safety goggles with side protection and protective gloves which meet the specification of standard norm EC directive 89/686 / EEC and the resultant standard EN374. Disposal considerations: Do not let product enter drains. Keep away from surface and ground water. Dispose of contents/containers must be in accordance with local, regional, national and international regulations.

NOTE: IS-pro™ eMix (Research 14007) contains Formamide. See MSDS and handle product accordingly!

Hazard statement code	Hazard statement	Hazard Class (GHS chapter)	Hazard Category
H351	Suspected of causing cancer	Carcinogenicity (chapter 3.6)	2
H360FD	May damage fertility. May damage the unborn child	Reproductive toxicity (chapter 3.7)	1A, 1B
H373	May cause damage to organs (blood, cardiovascular system) through prolonged or repeated exposure	Specific target organ toxicity, repeated exposure (chapter 3.9)	2

4.1 Components in IS-pro Microbiota kit

Mastermix FIRBAC (Research 14001)

3 vials with a blue colour insert, labelled "Mastermix Firbac" containing 132 µl ready to use PCR mix. This mixture contains five primers for amplification of the bacterial DNA of *Firmicutes/Actinobacteria/Fusobacteria/Verrucomicrobia* and *Bacteroidetes*. The mastermix contains all ingredients for PCR amplification including DNA polymerase.

Mastermix PROTEO (Research 14002)

3 vials with a yellow colour insert, labelled "Mastermix Proteo" containing 132 µl ready to use PCR mix. This mixture contains eight primers for amplification of the bacterial DNA of *Proteobacteria*. The mastermix contains all ingredients for PCR amplification including DNA polymerase.

Positive Control FIRBAC (Research 14003)

1 vial with a red colour insert, labelled "Positive Control Firbac" containing 30 µl positive control. The positive control contains bacterial DNA of *Firmicutes/Bacteroidetes*.

Positive Control PROTEO (Research 14004)

1 vial with a violet colour insert, labelled "Positive Control Proteo" containing 30 µl positive control. The positive control contains bacterial DNA of *Proteobacteria*.

IS-pro™ eMix (Research 14007)

2 vials with a black colour insert, labelled "IS-pro™ eMix" containing 540µl IS-pro™ eMix.

Note: Use all components of the same kit lot number.

4.2 Additional materials and instruments required

Use sterile DNase-free polypropylene disposables for all steps in the procedure.

IS-pro Microbiota kit can be used with standard guanidine iso-thiocyanate based lysis reagents and magnetic beads capture devices for DNA isolation. IS-pro Research kit is validated with DNA isolation in BioMérieux NucliSENS® easyMAG™, Chemagen chemagic MSM I instrument and Roche MagNA Pure 96 Instrument.

Validated sample types			
	NucliSENS® easyMAG™	Roche MagNA Pure 96 Instrument	Chemagen chemagic MSM I
High Load	Faeces Oral paperpoints/swabs Rectal swabs Cultured bacteria	Faeces Oral paperpoints <u>Respiratory samples:</u> <i>Bronchoalveolar lavage</i> <i>/Sputum/Nasopharyngeal aspirate</i>	Faeces Oral paperpoints
Low Load	Skin swabs		

PCR amplification can be carried out with standard PCR systems, e.g the PCR 9700 version 3.11 by Applied Biosystems. For separation and detection of the PCR amplicons an ABI Genetic Analyzer 3500 or 3130 is needed.

NOTE: The separation and detection process should be initiated within 12 hours after the PCR reaction is completed.

4.3 Reagent preparation and storage

Store all components at -20 °C.

Thaw the tubes prior to opening.
Mix and spin down briefly (3 seconds) before use.

All components are temperature sensitive.
Thaw only the components that are going to be used.
It is necessary to keep kit reagents at 2 – 8 °C (on ice or in cooling block) when in use.
Refreeze within half an hour after thawing.
Components can be refrozen and thawed again only twice.

4.4 Chemical or physical indications of instability

Alteration in the physical appearance of test kit materials may indicate instability or deterioration. Expiry dates shown on component labels indicate the date beyond which components should not be used.

5. Sample collection and preparation

The IS-pro Microbiota kit is intended to be used for detection and identification of bacteria for research use only.

General collection devices can be used for standard DNA isolation procedures according to the manufacturer's protocols.



**CAUTION: Handle patient samples as Biohazardous material.
Handle samples as if capable of transmitting an infectious agent.**

5.1 Sample Preparation

This procedure must be performed in the Sample Preparation Area in a class 2 Biological Safety Cabinet (protection to user and material).

Include a blank solution for every isolation run to control for contamination

5.1.1 Sample preparation validated for BioMérieux NucliSENS® easyMAG™:

Reagents

- BioMérieux NucliSENS® easyMAG™ reagents
- Gibco PCR grade water (Invitrogen article nr: 10977035)
- Transport Buffer (inBiome)

Sample preparation

Feces

- Add 100-400mg of feces (~0.5 cm diameter) to a vial.
- Add ~2 volumes of EasyMAG lysis buffer to 1 volume of feces, i.e. add 200 µL of lysis buffer to 100 mg feces
- Vortex the vial until feces is covered in EasyMAG lysis buffer at the bottom of the vial.
- Put the vial into a Mixer Incubator and shake and incubate at RT and 1400 rpm for 5 minutes.
- Centrifuge vial for 2 minutes at 18.000 rcf.
- Use 100 µL of supernatant for DNA isolation.

Oral swab, Rectal swab

- Add 1 mL of EasyMAG lysis buffer to the original sample vial containing the swab.
- Put the vial into a Mixer Incubator and shake and incubate at RT and 1400 rpm for 5 minutes.
- Centrifuge vial for 2 minutes at 18.000 rcf
- Use 200 µL of supernatant for DNA isolation.

Oral paperpoint

- 1mL of Transport buffer (inBiome Amsterdam) should be present in the transport vial to which paperpoints should be added.
- Put the vial into a Mixer Incubator and shake and incubate at RT and 1400 rpm for 5 minutes.
- Centrifuge vial for 10 seconds at 3500 rcf. This prevents aerosol formation when opening the container.
- Use 400 µL of supernatant for DNA isolation

Skin

- Add 500 µl of EasyMAG lysis buffer to the original sample vial containing the swab
- Put the vial into a Mixer Incubator and shake and incubate at RT and 1400 rpm for 5 minutes.
- Centrifuge vial for 10 seconds at 3500 rcf. This prevents aerosol formation when opening the container.
- Use complete volume for DNA isolation.

Cultured bacteria

- Add cultured bacteria to PCR grade water to make a 0.5 McFarland suspension.
- Use 200 µL for DNA isolation.

DNA isolation

For general usage of the EasyMAG see instruction manual of the machine.

Use one blank solution per EasyMAG isolation run, i.e. one of the wells is used for a blank solution. A blank solution is treated exactly like a bacterial sample without the actual specimen.

1. For each sample (including blanc solution):

- For each well of the EasyMAG container:
 - Add 2 ml of EasyMAG lysis buffer
 - Add pretreated material except to the blanc sample
 - Pipet 3 times up and down to mix the contents
 - Incubate at least for 10 minutes at RT. For pus/puncture samples extra incubation of 1 hour in EasyMAG lysis buffer at RT is necessary.
 - Remove visible particles with a sterile toothpick or by pipet
 - Add 70 μ l of EasyMAG magnetic silica
 - Mix the contents by pipetting

2. Start the isolation run as described in the EasyMAG manual using the following run conditions:

- Select the Specific A protocol with off-board lysis incubation
- For high bacterial load samples use 110 μ l eluate volume.
- For low bacterial load samples use 70 μ l eluate volume.

After isolation is completed, transfer DNA from EasyMAG container wells into the corresponding sterile vial for extracted DNA.

Store DNA at 2-8 °C

5.1.2 Sample preparation validated for Roche MagNA Pure 96 Instrument

Reagents

- PBS pH 7,4 (GIBCO, REF 10010)
- 'MagNA Pure' Lysis/binding buffer (Roche, MagNA Pure LC Total Nucleic Acid Isolation Kit 03 246 779 001)
- S.T.A.R. buffer (Roche 03 335 208 001)
- Proteinase K (Qiagen, REF 19133)

Sample preparation

Feces

- Add 1 mL S.T.A.R buffer in a 2 mL vial.
- Put a swab into the fecal sample. Note: make sure there is about 2mm feces on the swab.
- Break the swab at the breakdown point into vial filled with S.T.A.R. buffer.
- Put the vial overnight or minimal 1 hour into -80 °C freezer.
- Incubate the vial at 100 °C for 10 minutes.
- Cool down the vial and centrifuge for 10 seconds at 3500 rcf.
- Use 225 μ L for DNA isolation.

Oral Paperpoints

- Add 500 μ L PBS per sample
- Vortex the sample thoroughly
- Use 200 μ L for DNA isolation

Broncho-alveolaire lavage

- Add 300 µL of sample into a 2 mL vial.
- Check for flakes in the sample.
- If flakes are visible perform a Prot K pretreatment for MagNA Pure 96 on this vial.
- If no flakes are visible perform the liquid material sample preparation.

Sputum

- Check the sample on flakes and thickness.
- If flakes are visible, add 300 µL of sample into a 2 mL vial and perform a Prot K pretreatment for MagNA Pure 96 on this vial.
- If no flakes are visible perform the liquid material sample preparation.
- If the sputum is thick, add 1mL PBS to the material and vortex thoroughly. Deposit the sample and put 300 µL of supernatant into a 2 mL vial and perform a Prot K pretreatment for MagNA Pure 96 on this vial.

Nasopharyngeal swab

- Add 1mL PBS to the swabtube.
- Vortex the swab for minimal 5 minutes.
- Add 300 µL into a 2 mL vial and perform a Prot K pretreatment for MagNA Pure 96 on this vial.

Liquid materials

- Add 0,5 - 1 ml to a sterile vial and centrifuge for 10 minutes at 18.000 rcf
- Remove supernatant, leaving approximately 200 µl and resuspend in this 200 µl material.
- Perform a prot. K pretreatment for MagNA Pure 96 on this vial

Prot K pretreatment

- Add 20 µl Proteinase K to sterile vial.
- Add 200 µl MagNA Pure Lysis/binding buffer to sterile vial.
- Vortex for 10 seconds.
- Put the vial into a Mixer Incubator and shake and incubate at 56 °C and 1400 rpm until all tissue is lysed. The sample is lysed when no lumps are visible anymore. This takes approximately 1 hour. Incubation in Mixer Incubator can also be performed overnight at 37°C and 1400 rpm.
- Centrifuge the vial for 10 seconds at 3500 rcf.
- Incubate 10 minutes at 100°C in heating block
- Remove vials from heating block
- Incubate at RT for 3 minutes
- Centrifuge the vial for 10 seconds at 3500 rcf. This prevents aerosol formation when opening the container.
- Use complete volume for DNA isolation

DNA isolation

For usage of the MagNA Pure96 Instrument, see instruction manual of the machine.

Use one blank solution per 10 samples of the MagNA Pure96 isolation, at different positions in the plate. i.e. one or more of the wells of the plate is/are used for a blank solution depending on the sample size. A blank solution is treated exactly like a bacterial sample without the actual specimen.

5.1.3 Sample preparation validated for Chemagen chemagic MSM I Instrument.

Reagents

- Chemagic Lysis/binding buffer (Perkin Elmer, chemagic DNA Blood Kit special (Art.no. CMG-746) and chemagic DNA Buccal Swab Kit special (Art.no. CMG-748).

Sample preparation

Feces

- Add 200 mg of feces (~0.5 cm diameter) to a vial.
- Add 1,6 mL of Chemagen Lysis buffer.
- Vortex the vial and centrifuge for 10 seconds at 3500 rcf.
- Add 30 µl Protease, mix and incubate for 10 minutes at 70 °C using a thermo mixer at 1400rpm.
- Incubate another 5 minutes at 95 °C.
- Centrifuge for 5 minutes at 13000 rpm.
- Use 800 µl of the supernatant for DNA isolation.

Oral paperpoints

- Add 300 µl Lysisbuffer to the paperpoint and vortex thoroughly.
- Use 200 µL for DNA isolation.

DNA isolation

For usage of the Chemagen chemagic MSM I Instrument, see instruction manual of the machine.

Use the “Purification protocol for Buccal Swabs Using the chemagic Magnetic Separation Module I version 121018” for the DNA isolation of Oral paperpoints.

Use the “Purification Protocol for 250 µL of Human Blood Using the chemagic Magnetic Separation Module I version 120705”

Use two blank solution per 96 samples of the Chemagen chemagic MSM I isolation, at any positions in the plate. i.e. one or more of the wells of the plate is/are used for a blank solution depending on the sample size. A blank solution is treated exactly like a bacterial sample without the actual specimen.

5.2 DNA Isolated samples

This kit was validated with use of Nucleic Acid extracted samples by means of BioMérieux NucliSENS® easyMAG™, Chemagen chemagic MSM I instrument and Roche MagNA Pure 96 Instrument. Please follow the manufacturer’s instructions for a description of the system features, isolation protocols and operational guidelines.

Always isolate a blank solution. NB: The blank solution is a solution that is identical to the solution needed for sample preparation except that the blank solution does not contain analyte. The blank solution is used to identify contamination of samples.

6. IS-pro Microbiota kit Test Procedure

This procedure must be performed in the Pre-Amplification Preparation Area. Use aerosol barrier tips during the whole test procedure.

6.1 Reagent Preparation

Thaw the mixes needed and keep them at 0-8°C (on ice or a cooling block).

6.2 DNA pre-treatment

Bacterial DNA should be diluted in PCR grade water or Extractionbuffer 3 (BioMérieux NucliSENS® easyMAG™)

Bacterial DNA from fecal samples should be diluted in using a 1:10 ratio.

Bacterial DNA from cultured bacteria samples should be diluted in a 1:100 ratio.

Bacterial DNA from other sample types should be used undiluted.

6.3 Procedure PCR

1. Prepare the required number of reaction tubes or wells for the number of samples to be measured (two reactions per sample: Firbac and Proteo). NOTE: The first run of each kit should contain a Positive Control Firbac (Research 14003) and Positive Control Proteo (Research 14004), to validate machine set-ups. Handle positive controls as DNA.
2. Thaw and vortex Mastermix Firbac (Research 14001) and Mastermix Proteo (Research 14002). Add 15 µl of Mastermix Firbac (Research 14001) to the first reaction tube and 15 µl of Mastermix Proteo (Research 14002) to the second reaction tube.
3. Vortex and spin down all DNA extracts. Carefully open sample containers one by one and avoid contamination of gloves and pipette. Using a new aerosol barrier tip for each reaction tube. Add 10 µl DNA (Chapter 5) of each sample/blank to the reaction tube/well containing the Mastermix Firbac (Research 14001) and add 10 µl DNA of each sample/blank to the reaction tube/well containing the Proteo Mastermix (Research 14002). Replace gloves when contamination is suspected.
4. Close the reaction tubes or seal the plate, spin down for 10 seconds at approximately 1000 rpm and move the reaction tubes/plate to the Amplification Area.
5. Load the reaction tubes/plate into the PCR instrument: For detailed instructions on how to use the PCR machine refer to the PCR machine manual.
6. Program the PCR System with following settings:

PCR Protocols	
Low Load samples	High Load samples
10 cycles: <ul style="list-style-type: none">• 30 sec 94°C• 45 sec 67°C, reduce 1°C per cycle• 1 min 72°C 25 cycles: <ul style="list-style-type: none">• 30 sec 94°C• 45 sec 57°C• 1 min 72°C Final elongation and cooldown: <ul style="list-style-type: none">• 11 min 72°C• ∞ 4°C	35 cycles: <ul style="list-style-type: none">• 30 sec 94°C• 45 sec 56°C• 1 min 72°C Final elongation and cooldown: <ul style="list-style-type: none">• 11 min 72°C• ∞ 4°C

7. The PCR products can be stored for 12 hours at 2-8°C.

6.4 Procedure ABI

1. Thaw and vortex IS-pro™ eMix (Research 14007).
2. Per sample two PCR reactions were performed previously (Firbac and Proteo). For every sample, fill two wells of the ABI plate with 20 µl IS-pro™ eMix (Research 14007). To one well add 5 µl of the Firbac PCR product and to the other well 5 µl of the Proteo PCR product.
3. Spin down the plate for 10 seconds at approximately 1000 rpm.
4. Heat the plate in the PCR machine at 94°C for 3 minutes followed by a cooling step to 4 °C. Use the appropriate cover for the plate so no evaporation can occur.
5. Replace the plate-cover with ABI septa
6. Store plate at 2-8°C until capillary gel electrophoresis on the ABI. The ABI plate can be stored for five days at 2-8°C. The ABI is started using the ABI settings described below:

Instrument protocol ABI 3500				
Application type:	Capillary Length	Polymer	Dye Set	Advanced Options
Fragment	50 cm	POP7	D (DS-30)	unchanged from default settings

Instrument Protocol Properties								
Run Module	Oven Temperature	Run Time	Run voltage	PreRun Time	PreRun Voltage	Injection Time	Injection Voltage	Data Delay
FragmentAnalysis50_POP7	60°C	4500 sec	11.0 kVolts	180 sec	15 kVolt	15 sec	1.6 kVolts	200 sec

Dye Set protocol ABI 3500				
Dye Set Name	Chemistry Matrix	Dye Selection	Reduced Selection	Calibration Peak Order
D (DS-30)	Standard	Blue Green Yellow Red	Blue Green Yellow Red	Blue=4, Green=3, Yellow=2, Red=1

Parameters	
Matrix Condition Number Upper Limit	20.0
Locate Start Point	After Scan 300, Before Scan 5000, Limit Scans To 20000, Sensitivity 0.1, Minimum Quality Score 0.8

Sizecalling protocol ABI 3500	
Size Standard GS500ROX	
SizeCaller v1.1.0	

Analysis Settings											
Analysis Range	Sizing Range	Size Calling Method	Primer Peak	Minimum Peak Height	Use Smoothing	Use Baselining (Baseline window (Pts))	Minimum Peak Half Width	Peak Window Size	Polynomial Degree	Slope Treshold Peak Start	Slope Treshold Peak End
Full	Full	Local Southern	Present	All colors 175	None	51	2	15	3	0.0	0.0

QC Settings				
Fail if Value	Suspect Range	Pass if Value	Assume Linearity	Pull Up
< 0.25	0.25-0.75	≥ 0.75	0 bp To 800 bp	Actuate Pull-Up flag if Pull-Up Ratio ≤0.05 and Pull-Up Scans≤ 1

Instrument protocol ABI 3130 / ABI 3130XL setting I

Application type:	Capillary Length	Polymer	Dye Set	Advanced Options							
Fragment	50 cm	POP7	D (DS-30)	unchanged from default settings							

Instrument Protocol Properties

Run Module	Oven Temperature	Poly Fill Vol	Current Stability	PreRun Voltage	PreRun Time	Injection Voltage	Injection Time	Voltage Step Interval	Data Delay Time	Run Voltage	Run Time
FragmentAnalysis50_POP7	60°C	7300	5	15	180	3	15	11	200	15	4500

Instrument protocol ABI 3130 / ABI 3130XL setting II

Application type:	Capillary Length	Polymer	Dye Set	Advanced Options							
Fragment	36 cm	POP7	D (DS-30)	unchanged from default settings							

Instrument Protocol Properties

Run Module	Oven Temperature	Poly Fill Vol	Current Stability	PreRun Voltage	PreRun Time	Injection Voltage	Injection Time	Voltage Step Interval	Data Delay Time	Run Voltage	Run Time
FragmentAnalysis50_POP7	60°C	6500	5	15	180	3	23	11	60	11	4000

Instrument protocol ABI 3130 / ABI 3130XL setting III

Application type:	Capillary Length	Polymer	Dye Set	Advanced Options							
Fragment	36 cm	POP4	D (DS-30)	unchanged from default settings							

Instrument Protocol Properties

Run Module	Oven Temperature	Poly Fill Vol	Current Stability	PreRun Voltage	PreRun Time	Injection Voltage	Injection Time	Voltage Step Interval	Data Delay Time	Run Voltage	Run Time
FragmentAnalysis50_POP7	60°C	6500	5	15	180	3	23	11	60	11	4000

6.5 Analysing data

Various software packages are available to analyse your data. Examples include Peak Scanner and GeneMapper (Thermo Fisher Scientific) and 'Bionumerics (Applied Maths), or the **free** inBiome software service.

inBiome offers a free software service for analysing your data. The software handles all steps from raw data processing to peak calling and species identification. Fragments are assigned to bacterial species according to our database and translation algorithm. If the peaks are not identified by our database, a phylum name and fragment length will be assigned to each peak. Visualizations of profiles and interpretations of positive and negative controls are also available for users.

When users choose to work with the InBiome software service, then username and password should be requested via info@inBiome.com 10 working days before first usage of the kit.

When using the inBiome peak translation software service, it is advised to use the following naming convention:

INSTITUTION_DEPARTMENT_SAMPLETYPE_FIRBAC/PROTEO*_SAMPLENUMBER.fsa

Caution: Filenames must be anonymized before uploading to the software service, so they are not traceable to individuals.

*Choose FIRBAC for FAFV primergroup, choose PROTEO for Proteobacteria primergroup

6.6 Procedural notes

1. Use a uni-directional workflow in the laboratory.

Sample Preparation area: Dedicated area to prepare the samples. All materials (equipment, supplies, protection, gloves, etc.) have to be dedicated to this area. Materials from this area may not be moved to the Pre-Amplification area.

Pre-Amplification area: Dedicated area to prepare the reagents. All materials (equipment, supplies, protection, gloves, etc.) have to be dedicated to this area.

Amplification area: Dedicated area for amplification. All materials (equipment, supplies, protection, gloves, etc.) have to be dedicated to this area. Materials from this area, may not be moved to the Pre-Amplification Area, and may not be moved to the Specimen Preparation Area.

2. Always use aerosol resistant tips.
3. Be extremely careful when handling materials to prevent contamination. Always mix and spin down reagents and samples before opening. In case of any suspicion of contamination, discard the materials.
4. Discard all consumed reagents upon completion of procedure in compliance with local, regional, national and international waste regulations.
5. Careful analytical techniques and strict adherence to the directions in the test instructions are essential to obtain reliable results.
6. Samples with equivocal results must be verified by repeat assays or isolation.
7. Do not pool reagents from different lots.
8. If the kit is damaged upon receipt, please contact your local distributor and/or inBiome

7. Interpretation of results

The following dyes are used for the different targets:

Target	Dye
<i>Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia</i>	FAM
<i>Bacteroidetes</i>	HEX
<i>Proteobacteria</i>	FAM
Marker	ROX

Marker peaks are found at: 25, 50, 75, 100, 125, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1400 and 1500nc

8. Limitations of the procedure

1. Use only sample types mentioned. Other specimen types have not been validated and may result in false positive or false negative results.
2. Specimen collection, transport and storage may affect the number of organisms and their associated DNA present in the specimen, affecting the outcome of the result. (causing a false positive or a false negative result)
3. Good laboratory practices and strict adherence to these Test Instructions are indispensable to avoid contamination of reagents and/or specimens.
4. Bacteria from other phyla than *Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia Bacteroidetes* and *Proteobacteria* are not detected.
5. The user should have a formal training in PCR techniques or have gained appropriate experience in the field of PCR techniques.

9. References










Budding et al., (2010). IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota, FASEB J. 2010 Nov;24(11):4556-64.

Budding et al., (2014). Rectal swabs for Analysis of the intestinal Microbiota, PLoS ONE 2014 July; 9(7)e101344.

L.Daniels, A.E. Budding et al (2014). Fecal microbiome analysis as a diagnostic test for diverticulitis, Eur J Clin Microbiol Infect Dis. 2014 March, DOI 10.1007/s10096-014-2162-3

A.Eck et al. (2017). Robust Microbiota-Based Diagnostics for Inflammatory Bowel Disease, J. Clin. Microbiol. doi:10.1128/JCM.00162-17

List of symbols as used in labelling

Symbol	Meaning
	Manufacturer
	Catalogue number
	Batch code (Lot)
	Use by date
	Temperature limitation
	Contains sufficient for <n> tests
	Consult Instructions for Use
	Contents
	Danger. May damage fertility or the unborn child. Use personal protective equipment as required. If exposed or concerned: Get medical attention/advice. (Read MSDS)

List of Abbreviations

ABI	ABI Genetic Analyser 3500
DNA	Deoxiribonucleic acid
DNase	Desoxyribonuclease
EDTA	Ethylenediaminetetraacetic acid
FAFV	Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia
IS	Interspace
IS-pro™	Interspace Profiling
MSDS	Material Safety Data Sheet
NaCl	Sodium Chloride
Nc	Nucleotide
PC	Positive Control
PCR	Polymerase Chain Reaction
rDNA	ribosomal DNA
RNAse	Ribonuclease

WARRANTY

This product is warranted to perform as described in its labelling and in the inBiome literature when used in accordance with all instructions. inBiome DISCLAIMS ANY IMPLIED WARRANTY OF SPACE MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall InBiome be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser.

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