# Molecular Culture ID kit

Complies with the Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices

For In Vitro Diagnostic Use Only

## Test Instructions

**Amplification DNA assay for the semiquantitative in vitro detection of bacterial species from the phyla *Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia, Bacteroidetes,* and *Proteobacteria* in pus, punctates, drain fluids, ascites fluids, joint aspirates, and tissues from humans.**

Catalogue Number: MolCul 15000, 24 tests

**Store at -20°C upon receipt**

**Content**

## 1. Intended use

Molecular Culture ID is intended to be used for the detection and identification of bacteria isolated from pus, punctuates, drain fluids, ascites fluids, joint aspirates, and tissue samples, for use in clinical diagnostics in humans. Bacteria can be relatively quantified, based on their proportion of the total bacterial population within a specimen. The total bacterial signal in a sample is correlated to absolute quantification, which makes the results (i.e. bacterial species present in the sample) semi-quantitative.
The intended user will be a specialized molecular diagnostic laboratory. The test will be carried out by trained laboratory personnel. The test is semi-automated, as it requires some minor manual work on sample preparation pre- and inbetween automated processes. No special training will be required for professional routine diagnostic laboratories performing semi-quantitative PCR.

## 2. Product Description

Molecular Culture ID is based on the IS-pro™ technology. 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. Molecular Culture ID consists of two multiplex PCRs. The first PCR is specific for *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia,* and *Bacteroidetes*, the second PCR is specific for *Proteobacteria*. An internal PCR amplification control is used to control for PCR inhibition and efficiency.
A marker is added to the eMix as a length reference to determine the lengths of the PCR amplicons.

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

Molecular Culture ID can be used with standard guanidine iso-thiocyanate-based lysis reagents and magnetic beads capture devices for DNA isolation. Molecular Culture ID is validated for BioMérieux NucliSENS® easyMAG™.

*Validated sample types:*

| NucliSENS® easyMAG™ |
|---|
| Ascites fluid |
| Drain fluid |
| Joint aspirate |
| Punctate |
| Pus |
| Tissue |

PCR amplification can be carried out with any validated/verified PCR machine. See instructions for use Molecular Culture Starter Kit for validation. For separation and detection of the PCR amplicons, an ABI Genetic Analyzer 3500 and 3500XL are validated.

A uni-directional workflow in the laboratory is strongly recommended:
***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.

## 3. Molecular Culture ID kit components

### 3.1 Included in Molecular Culture ID kit

**Mastermix FIRBAC**
2 vials, labeled “Mastermix Firbac” containing 180 µl ready to use PCR mix. This mixture contains eight 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**
2 vials labeled “Mastermix Proteo” containing 180 µl ready to use PCR mix. This mixture contains eight primers for amplification of the bacterial DNA of *Proteobacteria* and contains DNA and primers for amplification of the internal control. The mastermix contains all ingredients for PCR amplification including DNA polymerase.

**Positive Control FIRBAC**
2 vials labeled “Positive Control Firbac” containing 30 µl positive control. The positive control contains the bacterial DNA of *Firmicutes/Bacteroidetes*.

**Positive Control PROTEO**
2 vials labeled “Positive Control Proteo” containing 30 µl positive control. The positive control contains the bacterial DNA of *Proteobacteria*.

**eMix**
1 vial labeled “eMix” containing 480 µl eMix.

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

### 3.2 Not included in Molecular Culture ID kit

- Input DNA
- PCR machine
- ABI 3500 XL machine, ABI 3500 machine, SeqStudio machine, or SeqStudio Flex machine
- Computer and web browser, connected to the internet

## 4. Storage

- Molecular Culture ID is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, please contact inbiome.
- All components should be stored at -20°C upon arrival.
- Repeated thawing and freezing of Mastermixes (more than nine times), positive control (more than nine times), and eMix (more than nine times) should be avoided, as this might affect the performance of the assay.
- Refreeze within half an hour after thawing.
- Protect all components from light.
- 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.
- Mastermix and control vials should be stored in a ***Sample Preparation area*** or ***Pre-Amplification area***
- eMix can be stored in the ***Amplification area***

## 5. Warnings and 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.

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

Use sterile, DNase-RNase-free, aerosol resistant pipette tips and wear protective gloves. Protect kit contents or generated PCR product from direct sunlight.

For handling eMix 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 groundwater. Disposal of contents/containers must be following local, regional, national, and international regulations.

**NOTE: eMix (MolCul 15007) contains Formamide. See MSDS and handle product accordingly!**

**Precautionary Statements**
P308 + P313 - IF exposed or concerned: Get medical advice/attention
P202 - Do not handle until all safety precautions have been read and understood
P260 - Do not breathe dust/fume/gas/mist/vapours/spray
P201 - Obtain special instructions before use
P281 - Use personal protective equipment as required
P314 - Get medical advice/attention if you feel unwell

NOTICE: any serious incident that has occurred in relation to the device must be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

| Classification eMix according to regulation (EC) No 1272/2008 (CLP) | | | | | |
|---|---|---|---|---|---|
| **Section** | **Hazard Class** | **Category** | **Hazard class and category** | **Hazard statement code** | **Hazard statement** |
| 3.6 | Carcinogenicity | 2 | Carc. 2 | H351 | Suspected of causing cancer |
| 3.7 | Reproductive toxicity | 1B | Repr. 1B | H360F | May damage fertility, may damage the unborn child |
| 3.9 | Specific target organ toxicity, repeated exposure | 2 | STOT RE 2 | H373 | May cause damage to organs (blood, cardiovascular system) through prolonged or repeated exposure |

## 6. Sample collection and preparation

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

### 6.1 Sample preparation validated for BioMérieux NucliSENS® easyMAG™:

**Reagents**

- Bacterial Shock Buffer 1 (IBB23000, inbiome)
- Bacterial Shock Buffer 2 (IBB24000, inbiome)
- BioMérieux NucliSENS® easyMAG™ reagents
- Zirconia/Silica beads 0.1mm (11079101Z, BioSpec)
- Qiagen AL buffer (19075, Gibco PCR grade water (10977035, Invitrogen)

**Sample preparation**

Biopsies

- Add to the sterile vial containing 500 µl of Bacterial Shock Buffer 1 and 400 mg of Zirconia/Silica beads 0.1mm the sample, size of a pinhead.
- Incubate for 10 minutes at 800 rpm by 95 °C in thermomixer: check for homogeneity of the sample.
- Add 50 µl Bacterial Shock Buffer 2 and vortex.
- Spin down shortly.
- Beadbeating: 1x 180 seconds at room temperature.
- Spindown and collect the supernatant.
- Add 1 ml easyMAG™ lysisbuffer + 1 ml AL buffer to an easyMAG™ vessel.
- Add pretreated material except to the blanc sample. (Use the complete volume of supernatant for DNA isolation)

Synovial fluid / joint aspirate

- Add to the sterile vial 250 µl of Bacterial Shock Buffer 1 and 50 µl sample.
- Vortex the samples.
- Incubate 10 minutes at at 95 °C 800 rpm in thermomixer: check for homogeneity of the sample.
- Add 25 µl Bacterial Shock Buffer 2 and vortex.
- Spin down shortly.
- Add 1 ml easyMAG™ lysisbuffer + 1 ml AL buffer to the easyMAG™ vessels.
- Add pretreated material to the easyMAG™ except to the blanc sample. (Use the complete volume of supernatant for DNA isolation)

Pus sample, puncture sample, drain fluid (ascites fluid)

- Add to the sterile vial 250 µl of Bacterial Shock Buffer 1 and 200 µl sample.
- Vortex the samples.
- Incubate 10 minutes at at 95 °C 800 rpm in thermomixer: check for homogeneity of the sample.
- Add 25 µl Bacterial Shock Buffer 2 and vortex.
- Spin down shortly.
- Add 1 ml easyMAG™ lysisbuffer + 1 ml AL buffer to the easyMAG™ vessels.
- Add pretreated material to the easyMAG™ except to the blanc sample. (Use the complete volume of supernatant for DNA isolation)

**Machine protocol**

Please follow the manufacturer's instructions for a description of the usage of the easyMAG™ machine, system features, isolation protocols, and operational guidelines.

For each easyMAG™ vessel:

- Pipet 3 times up and down to mix the contents.
- Incubate at least for 10 minutes at room temperature.
- Remove visible particles with a sterile toothpick or by pipet.
- Add 70 ul of easyMAG™ magnetic silica.

- Mix the contents by pipetting.
- 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.
  - Use 70 µl eluate volume.

Store DNA at 2-8 °C.

## 7. Molecular Culture ID Kit Test Procedure

This procedure must be performed in the Pre-Amplification Preparation Area. Use aerosol barrier tips during the whole test procedure. Thaw only the components that are going to be used. Mix and spin down reaction tubes briefly (three seconds) before use.

### 7.1 Procedure PCR

1. Prepare the required number of reaction tubes or wells for the number of samples to be measured. Two reactions per sample (and controls) should be prepared. One Firbac and one Proteo reaction. NOTE: The first run of each kit should contain a Positive Control Firbac and Positive Control Proteo. Handle positive controls as DNA.

2. Thaw and vortex Mastermix Firbac and Mastermix Proteo. Per sample, add 15 µl of Mastermix Firbac to the first reaction tube and 15 µl of Mastermix Proteo to the second reaction tube.

3. Vortex and spin down all DNA extracts. Add 10 µl DNA (Chapter 6) of each sample to the reaction tube/well containing Mastermix Firbac and add 10 µl DNA of each sample to the reaction tube/well containing Mastermix Proteo.

4. Close the reaction tubes or seal the plate, spin down for 30 seconds at approximately 1000 rpm.

5. Place the reaction tubes/plate into the for Molecular Culture varified thermocycler and program the system as listed below:

| PCR Protocol | |
|---|---|
| **Hotstart** | 10 min 95˚C |
| **10 cycles** | 30 sec 95˚C |
| | 45 sec 67˚C, reduce 1˚C per cycle |
| | 1 min 72˚C |
| **25 cycles** | 20 sec 95˚C |
| | 30 sec 57˚C |
| | 30 sec 72˚C |
| **Final elongation and cooldown** | 2 min 72˚C |
| | ∞ 4˚C |

6. The PCR products can be stored for 12 hours at 2-8˚C in the dark.

### 7.2 Procedure Capillary Electrophoresis

1. Thaw and vortex eMix.

2. Per sample, two PCR reactions were performed previously (Firbac and Proteo). For every sample, fill one well of the ABI plate with 20 µl eMix (MolCul 15007). Add 2,5 µl of the Firbac PCR product and 2,5 µl of the Proteo PCR product. NB: The Positive Control Firbac and Positive Control Proteo can be pipetted together as one sample.

3. Spin down the plate for 30 seconds at approximately 1000 rpm.

4. Put the plate in the thermocycler 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 electrophoresis. The plate can be stored for five days at 2-8°C in the dark. Capillary electrophoresis is started using the settings described in the tables below:

7. Start the Capilary electrophoresis according the settings set for the for Molecular Culture verified CE-machine. Settings are shown per instument in the tables: ‘ Settings ABI 3500’ , ‘Settings ABI3500XL’, ‘Settings Seqstudio’, ‘Settings Seqstudio Flex’.

**Settings ABI 3500**

| Instrument protocol ABI 3500 | | | | |
|---|---|---|---|---|
| Application type: | Capillary Length | Polymer | Dye Set | Advanced Options |
| Fragment | 50 cm | POP7 | G5 | 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 | 3 kVolts | 200 sec |

| Dye Set protocol ABI 3500 | | | | |
|---|---|---|---|---|
| Dye Set Name | Chemistry | Dye Selection | Reduced Selection | Calibration Peak Order |
| G5 | Matrix Standard | Blue Green Yellow Red Orange | Blue Green Yellow Red Orange | Blue=5, Green=4, Yellow=3, Red=2, Orange=1 |

| Parameters | |
|---|---|
| Matrix Condition Number Upper Limit | 13,5 |
| Locate Start Point | After Scan 1000, Before Scan 5000, Limit Scans To 3250, Sensitivity 0.4, Minimum Quality Score 0.95 |

| Sizecalling protocol ABI 3500 * | |
|---|---|
| Protocol name | Fragment Analysis PA_Protocol |
| Size Standard | GS600LIZ (60-600) + Normalisation |
| Sizecaller | 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 |
| Full | Full | Local Southern | Present | All colors 175 | None | 51 | 2 | 15 | 3 | 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 Scan≤ 1 |

* Molecular Lab Cloud /antoni (inbiome's free software service) does not require a sizecalling protocol because it uses raw data from the .fsa . but since sizecalling is obliged in the instrument software please use the sizecalling protocol above.

**Settings ABI 3500XL**

| Instrument protocol ABI 3500XL | | | | |
|---|---|---|---|---|
| Application type: | Capillary Length | Polymer | Dye Set | Advanced Options |
| Fragment | 50 cm | POP7 | G5 | 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 | 45 sec | 3 kVolts | 200 sec |

| Dye Set protocol ABI 3500XL | | | | |
|---|---|---|---|---|
| Dye Set Name | Chemistry | Dye Selection | Reduced Selection | Calibration Peak Order |
| G5 | Matrix Standard | Blue Green Yellow Red Orange | Blue Green Yellow Red Orange | Blue=5, Green=4, Yellow=3, Red=2, Orange=1 |

| Parameters | |
|---|---|
| Matrix Condition Number Upper Limit | 13,5 |
| Locate Start Point | After Scan 1000, Before Scan 5000, Limit Scans To 3250, Sensitivity 0.4, Minimum Quality Score 0.95 |

| Sizecalling protocol ABI 3500XL * | |
|---|---|
| Protocol name | Fragment Analysis PA_Protocol |
| Size Standard | GS600LIZ (60-600) + Normalisation |
| Sizecaller | 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 |
| Full | Full | Local Southern | Present | All colors 175 | None | 51 | 2 | 15 | 3 | 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 |

* Molecular Lab Cloud /antoni (inbiome's free software service) does not require a sizecalling protocol because it uses raw data from the .fsa . but since sizecalling is obliged in the instrument software please use the sizecalling protocol above.

**Settings SeqStudio Flex**

| Instrument protocol SeqStudio Flex | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Application type: | Capillary Length | Polymer | Dye Set | | | | | |
| Fragment analysis | 50 cm | POP7 | G5 | | | | | |
| **Instrument Protocol Properties** | | | | | | | | |
| Run Module | Oven Temperature | Run Time | Run voltage | PreRun Time | PreRun Voltage | Injection Time | Injection Voltage | Data Delay |
| adjusted from FragmentAnalysis50_ POP7 | 60°C | 4500 sec | 11.0 kVolts | 180 sec | 15 kVolt | 45 sec | 3 kVolts | 200 sec |

**Settings SeqStudio**

| Instrument protocol SeqStudio | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Application type: | Dye Set | | | | | | | | |
| Fragment analysis | G5 | | | | | | | | |
| **Instrument Protocol Properties** | | | | | | | | | |
| Run Module | Capillary Temperature | Run Time | Run voltage | Run Ramp Duration | PreRun Time | PreRun Voltage | Injection Time | Injection Voltage | Data Delay |
| adjusted from LongFragAnalysis | 60°C | 5960 sec | 4 kVolts | 300 sec | 180 sec | 13 kVolt | 14 sec | 1.2 kVolts | 350 sec |

### 7.3 Analysing data

Analyzing data should be performed according to the instructions provided in section 8.

Various software packages are available to analyse your data. Examples include Peak Scanner and GeneMapper (Thermo Fisher Scientific) and Bionumerics (Applied Maths), or Molecular Lab Cloud / antoni.

inbiome offers Molecular Lab Cloud / antoni (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 Molecular Lab Cloud / antoni, and they do not yet have an account, then username and password should be requested via sales@inbiome.com.

With Molecular Lab Cloud >250 bacterial species from the phyla *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia*, *Bacteroidetes* and *Proteobacteria* can be identified. Species that are not in the current database are identified to this phylum level. The database is regularly updated. The most recent list of identifiable species can be found at www.inbiome.com/molecular-culture

### 7.4 Procedural notes

1. 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.
2. Careful analytical techniques and strict adherence to the directions in the test instructions are essential to obtain reliable results.
3. Samples with equivocal results must be verified by repeat assays or isolation.
4. Do not pool reagents from different lots.
5. If the kit is damaged upon receipt, please contact your local distributor and/or inbiome.

## 8. Interpretation of results

The following dyes are used for the different targets:

| Target | Dye |
|---|---|
| ***FAFV*** | FAM |
| ***Bacteroidetes*** | VIC |
| ***Proteobacteria*** | NED |
| **Internal Control** | PET |
| **Marker** | LIZ |

Marker fragments 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

For valid runs (Valid Positive and Negative Control – See Quality Control), interpret the specimen results as follows:

**Quality control**
In each kit positive controls for the FIRBAC and PROTEO reaction are provided. Additional controls may be analysed in addition to those provided. Established statistical methods for analyzing control values and trends should be employed.

If the controls do not comply with the established limits and repetition excludes a technical issue, check the following areas:

1. The expiration date on the reagent package and prepared reagents
2. The temperature of the reagents
3. Settings PCR System
4. Settings ABI System
5. Contamination

If controls are still invalid, please contact inbiome or your local distributor. **Note:** following criteria are obtained with Molecular Lab Cloud/antoni.

*Positive Control Firmicutes*
Fragment length should be at 299-301 nucleotides and the intensity should be at least 15 000 RFU. If these criteria are not met, the complete run is invalid and the test procedure must be repeated.
*Positive Control Bacteroidetes*
The true peak positionFragment length should be at 535-537 nucleotides and the intensity should be at least 15 000 RFU. If these criteria are not met, the complete run is invalid and the test procedure must be repeated.

*Positive control Proteobacteria*
The true peak positionFragment length should be at 856-858 nucleotides and the intensity should be at least 15 000 RFU. If these criteria are not met, the complete run is invalid and the test procedure must be repeated.

*Internal control*
Internal control fragments should be at positions 503 +/- 1 nc and 1125 nc +/- 3 nc. 2 out of 2 peaks should be detected per sample or one proteo peak should be present higher then 15.000 RFU between nucleotide 145 and 1500. If these criteria are not met, the sample is invalid and the test procedure must be repeated.

*Negative Control*
All peaks > 145 nc should be below 4000 RFU. If these criteria are not met, the negative control is contaminated.

## 9. Limitations of the procedure

1. Use only specimens described in the intended use. 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. Bacteria from other phyla than *Firmicutes*, *Actinobacteria*, *Fusobacteria*, *Verrucomicrobia Bacteroidetes* and *Proteobacteria* are not detected.

4. Good laboratory practices and strict adherence to these Test Instructions are indispensable to avoid contamination of reagents and/or specimens.

## 10. Performance characteristics

All results of the following performance characteristics have been obtained with Molecular Lab Cloud:

## 10.1 Analytical specificity

**Cross-reactivity with non-bacterial organisms**

The analytical specificity of the Molecular Culture ID kit was tested against cultured *Candida albicans, Cryptococcus neoformans/neoformans, Malassezia furfur, Absidia corymbifera, Rhizomucor pusillus, Rhizopus oryzae, Aspergillus fumigatus, Fusarium oxysporum, Saccharomyces cerevisiae, Trichosporon asashii, Blastocystis hominus*, and *Cytomegalovirus*. All tested organisms gave negative results, indicating no cross-reactivity with DNA from the tested cultured organism.

**Cross-reactivity between the three different phyla (FAFV group, *Bacteroidetes,* and *Proteobacteria*)**

Cross-reactivity between *Firmicutes* (*Clostridium tertium, Peptoniphilus assacharolyticus, and Staphylococcus haemolyticus)* and *Proteoprimers* could be observed when the DNA concentration was very high (cultured bacteria), which gives the primers a chance to bind aspecifically. The *Proteopeaks* are only created when the Firmicutes peak is also present. No bacterial signal was detected in other channels than expected for *Bacteroidetes* and *Proteobacteria.* In practice, the risk of cross-reactivity between the three different phyla is very low, but if it happens in clinical samples it may be used as additional information to identify a bacterial species.

**Cross-reactivity with human DNA**

Cross-reactivity with human DNA can occur when human DNA load is high and bacterial DNA absent or in very low concentrations relative to human DNA. Human cross-reactivity peaks may occur alone or in various combinations. The freely available Molecular Lab Cloud can automatically detect and delete such fragments.

## 10.2 Analytical sensitivity

The **limit of detection** (LoD) of the Molecular Culture ID kit was determined using quantified stock cultures (by counting CFU). The table below shows the determination of the LoD with at least 95% confidence.

*Limit of Detection per phylum*

| Phylum | Bacteria | LoD | Confidence |
|---|---|---|---|
| ***Firmicutes*** | *Streptococcus cristatus* (DSM 8249T / ATCC 13637) | 5 CFU | >95% |
| ***Firmicutes*** | *Streptococcus bovis* (IS-I, clinical sample, no strain known) | 5 CFU | >95% |
| ***Bacteroidetes*** | *Bacteroides fragilis* (NCTC 9343 / DSM 2151) | 1 CFU | >95% |
| ***Bacteroidetes*** | *Bacteroides thetaiotaomicron* (VPI 5483/DSM 2079) | 5 CFU | >95% |
| ***Proteobacteria*** | *Stenotrophomas maltophilia* (DSM 50170 / ATCC 13637) | 5 CFU | >95% |
| ***Proteobacteria*** | *Pseudomonas aeruginosa* (ATCC 27853) | 5 CFU | >95% |

To determine the **dynamic range** of a bacterium in the presence of another bacterium from the same phylum. The dynamic range of the FAFV group was 1:100, the dynamic range of the *Bacteroidetes* was 1:1000 and the dynamic range of the *Proteobacteria* was 1:1000. For the overall dynamic range the lowest measured dynamic range was taken, 1:100.

## 10.3 Precision

The **repeatability** (within run values) was tested with one bacterium per phylum in 15 fold. Results were within the range.

*Repeatability of positive control per phylum log2 transformed*

| | -2StDev (log2intensity) | -1StDev (log2intensity) | Average (log2intensity) |
|---|---|---|---|
| ***Firmicutes*** | 14,06 | 14,42 | 14,78 |
| ***Bacteroidetes*** | 13,36 | 13,94 | 14,53 |
| ***Proteobacteria*** | 13,94 | 14,34 | 14,74 |

*Repeatability of 10 CFU bacterial analyte per phylum log2 transformed*

| | -2StDev (log2intensity) | -1StDev (log2intensity) | Average (log2intensity) |
|---|---|---|---|
| ***Firmicutes*** | 13,64 | 14,10 | 14,56 |
| ***Bacteroidetes*** | 12,17 | 12,9 | 13,62 |
| ***Proteobacteria* + IC** | 11,26 | 12,17 | 13,07 |

For the **reproducibility**, the effects of the variables were evaluated for two values (different run dates, different operators, different PCR machines, and different ABI machines). Different variables were tested simultaneously. Results

were within the range of 2SD (0.93 log2RFU) which has been set as a reference for reproducibility as described in 'IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota.

### 10.4 Accuracy

The systematic error (trueness of results) is defined as the performance of the Molecular Culture ID kit relative to culture (golden standard).

In a recent study in which 591 joint aspirate samples were tested , a total of 177 samples were positive by culture, and 213 were positive with Molecular Culture ID. 8 samples were positive with culture and negative with Molecular Culture ID. These were all likely contaminated samples with low loads of skin commensals. 44 samples were positive with Molecular Culture ID and negative with traditional culture. In most cases, clinical contest supported these additional findings. In conclusion, Molecular Culture ID outperformed traditional culture for diagnostics of infections. Combined sensitivity for Molecular Culture ID was 96% (213/221), and 80% (177/221) for traditional culture.

The entire dataset of this study can be requested via sales@inbiome.com

### 10.5 Interfering Substances

The presence of PCR inhibitors may cause false negative results. The tables below show the effect of possible interfering compounds.

| Possible interfering compound | Interference with Firbac PCR | Interference with Proteo PCR |
|---|---|---|
| **Metronidazole** | No | Yes |
| **Magnetic Silica** | No | Yes |
| **DNAse/RNAse free water** | No | No |

| Possible Interfering compound | Dilution factor without interference Firbac PCR | Dilution factor without interference Proteo PCR |
|---|---|---|
| **EDTA** | 10x | 10x |
| **Biomerieux Lysis buffer** | 100x | *All tested dilutions interfered with Proteo PCR* |
| **96% EtOH** | 10x | 100x |
| **NaCl** | 10x | 100x |

### 10.6 Assay list

| Assay Performance | |
|---|---|
| **Dynamic Range** | 1:100 |
| **Precision** - Repeatablility | Positive control, -2*stdev: *Firmicutes*: 14.06 (log2intensity), *Bacteroidetes*: 13.36 (log2intensity), *Proteobacteria*: 13.94 (log2intensity)<br>Positive control, -1*stdev: *Firmicutes*: 14.42 (log2intensity), *Bacteroidetes*: 13.94 (log2intensity), *Proteobacteria*: 14.34 (log2intensity)<br>10 CFU, -2*stdev: *Firmicutes*: 13.64 (log2intensity), *Bacteroidetes*: 12.17 (log2intensity), *Proteobacteria*: 11.26 (log2intensity)<br>10 CFU, -1*stdev: *Firmicutes*: 14.1 (log2intensity), *Bacteroidetes*: 12.9 (log2intensity), *Proteobacteria*: 12.17 (log2intensity) |
| **Precision** - Reproducibility | 2SD log2RFU(0.93) for 95% of the peaks |
| **Cross Reactivity** - Bacterial species of other Phyla | No cross reactivity except for selected species |
| **Cross Reactivity** - Human DNA | Cross reactivity in high human DNA load samples have been identified. |
| **Interference** - Amplification inhibitors | Preferably monitored by internal amplification control |
| **Sensitivity** - Analytical | At least 95% sensitivity compared to culture for species included in assay |
| **Sensitivity** - Functional (peak position (nc)) | ±1nc |
| **Sensitivity** - Limit of Blank (RFU) | Intensity < 4000 |
| **Sensitivity** - Limit of Detection (RFU) | 1 to 5 CFU (>95% confidence). |
| **Correlation study** - Reference method | Culture |
| **Correlation study** - Reference standard | Clinical samples |

| Assay Protocol | |
|---|---|
| **Sample pretreatment** | Lysis + DNA isolation, (Lysis + DNA isolation reagents are not included in the kit) |
| **Reagent pretreatment** | NA |
| **Type of sample** | DNA derived from pus, punctates, drain fluids, ascites fluids, joint aspirates, tissues from humans. |
| **Sample size limitations (w/o dead volume)** | NA |
| **Assay format** | Thermocycler, ABI3500 (XL), ABI SeqStudio, ABI SeqStudio Flex |
| **Automatic/Manual Dilution** | NA |
| **Total assay time** | <5 hr |
| **Reagents, Calibrators and Controls** | |
| **Tests per kit** | 24 |
| **Positive control** | |
| A. Matrix | Liquid frozen inactivated DNA |
| B. Physical form | Liquid frozen |
| C. Stability (shelf life)* | 12 months |
| D. Number of freeze/thaw cycles | 9 |
| E. Stability after defrosting (2-8°C) | 30 minutes between every freeze/thaw step |
| **Mastermix** | |
| A. Matrix | Liquid frozen with Taq Polymerase and no DNA control |
| B. Physical form | Liquid frozen |
| C. Stability (shelf life) | 12 months |
| D. Number of freeze/thaw cycles | 9 |
| E. Stability after defrosting (2-8°C) | 30 minutes between every freeze/thaw step |
| **Shipping conditions** | On dry ice |
| **Storage conditions** | -20°C |
| **Legal/Regulatory** | |
| **Intended use** | Molecular Culture ID is intended to be used for the detection and identification of bacteria isolated from pus, punctuates, drain fluids, ascites fluids, joint aspirates, and tissue samples, for use in clinical diagnostics in humans. Bacteria can be relatively quantified, based on their proportion of the total bacterial population within a specimen. The total bacterial signal in a sample is correlated to absolute quantification, which makes the results (i.e. bacterial species present in the sample) semi-quantitative.<br>The intended user will be a specialized molecular diagnostic laboratory. The test will be carried out by trained laboratory personnel. The test is semi-automated, as it requires some minor manual work on sample preparation pre- and inbetween automated processes. No special training will be required for professional routine diagnostic laboratories performing semi-quantitative PCR. |

## 11. 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., (2016). Automated broad range molecular detection of bacteria in clinical samples, JClin Microbiol. 2016 Apr;54(4):934-43.

**List of symbols as used in labeling**

| | |
|---|---|
|  | Manufacturer |
|   | Catalogue number |
|   | Batch code (Lot) |
|  | Expiration 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** |
| **DNA** | **Deoxiribonucleic acid** |
| **DNAse** | **Desoxyribonuclease** |
| **EDTA** | **Ethylenediaminetetraacetic acid** |
| **FAFV** | **Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia** |
| **IC** | **Internal Control** |
| **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 labeling 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.

THE PURCHASE OF THIS PRODUCT GRANTS THE PURCHASER RIGHTS UNDER CERTAIN INBIOME PATENTS TO USE IT SOLELY FOR PROVIDING HUMAN IN VITRO DIAGNOSTIC SERVICES. NO GENERAL PATENT OR OTHER LICENCE OF ANY KIND OTHER THAN THIS SPECIFIC RIGHT OF USE FROM PURCHASE IS GRANTED HEREBY.

DISCLAIMER

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

TECHNICAL ASSISTANCE

For additional information, please visit www.inbiome.com

For technical assistance please refer to the Catalogue Number: Molcul 15000

Manufacturer: inbiome
Science Park 106
1098 XG Amsterdam
The Netherlands
Tel: +31 (0)20 – 2380320