# MutaGEL® Parodontitis

Immundiagnostik AG, D-64625 Bensheim, Stubenwald-Allee 8a, Tel: +49(0)6251-70190-0, Fax: +49(0)6251-849430, www.immundiagnostik.com

## 1. Intended Use

**Code: KE09006**

The test kit "MutaGEL® Parodontitis" allows the detection of a selection of up to three from the five major pathogens (*Porphyromonas gingivalis, Tannerella forsythensis, Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans* and *Prevotella intermedia*) leading to chronic parodontitis, agressive juvenile parodontitis and necrotic ulcerative gingivitis. Matrix for this analysis is bacteria-DNA isolated from gingival pocket.

## 2. Introduction

Parodontitis is the main cause of tooh loss in adults. The mucous membranes of healthy human cavity of the mouth are colonized with billions of microorganisms. The different species (about 300) secrete growth factors and inhibitors thereby regulating their own number. This ecological system of microorganisms is in permanent steady state. Beside the physiological bacteria also pathogenic organisms will grow up in the mouth (more precisely in the gingival pocket). An excessive growth of these pathogens disturbs the physiological balance and favours development of parodontitis (making a therapy with antibiotics necessary). Once started, parodontitis leads in its further course to slow degradation of the parodontium. This stage begins mostly at the age of 30-35 years resulting in first teeth loss at the age of 40 years (if no treatment occurs).

General **diagnostic questions** are: **A) chronic parodontitits** (*Porphyromonas gingivalis, Tannerella forsythensis and also Fusobacterium necrotum*), **B) aggressive parodontitis** in younger patients (*Actinobacillus actinomycetemcomitans*) + **C) necrotic ulcerative ginigivitis** (*Prevotella intermedia*).

## 3. Principle of the Test

The kit „MutaGEL® Parodontitis" contains different sets of primer which amplify each a specific sequence of the following pathogens: *Porphyromonas gingivalis, Tannerella forsythensis, Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans* and *Prevotella intermedia*. Additionally, with primers for a highly conserved region of the bacterial 16S-RNA gene, an optical quantification of all contained bacteria can be done (analogous to the classical determination of germ numbers). In dependence of the diagnostic question the required primers for each individual case must be chosen before performance (provided Master Mix is enough to perform up to 96 PCRs and each pathogen specific primer mix allows 24 PCRs). Subsequently, the amplified pathogens are detected by classical gel electrophoresis (Pipetted in parallel. The generated amplicons have all different sizes). Development of this PCR method by Dr. Essrich, Biologisches Labor Freiburg/ Denzlingen (Germany).

In case of individual needs, **Immundiagnostik may provide a selected combination of primer pairs** in order to create an **adaption to your specific laboratory requirement**. Please contact us (your distributor) for such an individual kit!

## 4. Material Supplied (24 for a selected diagnostic question, 96 PCRs all together)

| | | | |
|---|---|---|---|
| ▪ Primer **TF** (Tannerella forsythensis) | 1 x | 70 µl (white) | reagents with oligonucleotids, specific for one specific bacterium bacterium leading to parodontitis |
| ▪ Primer **PG** (Porphyromonas gingivalis) | 1 x | 70 µl (**brown**) | " |
| ▪ Primer **AA** (Actinobacillus actinomycetemcomitans) | 1 x | 70 µl (**violet**) | " |
| ▪ Primer **PI** (Prevotella intermedia) | 1 x | 70 µl (**green**) | " |
| ▪ Primer **FN** (Fusobacterium nucleatum) | 1 x | 70 µl (**blue**) | " |
| ▪ Primer **16** (bacterial 16S-RNA) | 1 x | 70 µl (transp.) | oligonucleotids specific for bacterial 16S-RNA as IC |
| ▪ PCR-Master Mix (ready to use), without primer | 2 x | 850 µl (**yellow**) | ready to use PCR Mix (*hot start* Taq, $MgCl_2$, dNTPs, buffer) |
| ▪ Positive control DNA | 1 x | 70 µl (**red**) | buffered solution with amplified DNA from all the parodontitis germs (except *Prevotella intermedia,* **PI**) |
| ▪ Negative control | 1 x | 100 µl (transp.) | Tris buffer for no template control (NTC) |

## 5. Materials Required but not Supplied

Reagents and instruments:

- DNA extraction kit, suited for gram-positive bacteria (e.g. MutaCLEAN® Bacteria, KE5096, Immundiagnostik).
- thermal cycler and mineral oil (optional, for thermocycler without heated lid)
- pipettes (0.5 - 200 µl) and sterile pipette tips
- sterile micro tubes suitable for the thermal cycler in use
- reagents and instruments for gel electrophoresis

## 6. Storage and Stability

Store at ≤ -18°C. The reagents are stable in the unopened micro tubes until the expiration date indicated (see print on the package). Do not thaw out the content of the positive control DNA for more than two times. If necessary, make suitable aliquots.
*Before use*: Spin tubes briefly before opening (contents may become dispersed during shipment).

## 7. Warning and Precautions

- For in vitro diagnostic use only.
- Test should only be performed only by skilled persons considering GLP (Good Laboratory Practice) guidelines.
- Don't use the kit after its expiration date.
- After usage, dispose all reagents and test components included in the kit in conventional garbage.
- PCR technology is extremely sensitive. The amplification of a single DNA molecule generates million identical copies. Therefore set up three separate working areas for a) sample preparation, b) PCR reagent preparation and c) DNA detection. For each working area a different set of pipettes should be reserved.
- Wear separate coats and gloves in each working area and avoid aerosols.
- Use sterile filter tips for pipetting and use special PCR pipettes for aerosol free pipetting.
- Routinely decontaminate your pipettes and the laboratory benches.

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## Procedure

The complete procedure is divided in three steps:

1. Sample preparation.
2. Amplification with primers specific for pathogens leading to parodontitis.
3. Detection of the amplified DNA by gel electrophoresis.

## 8. Sample Preparation

- Extract bacterial DNA (from 200 µl of gingival pocket lavage) using a commercial available DNA isolation kit (suitable also for lysation of gram-positive microorganisms) according to the manufacturer`s instructions.
- Start immediately with the amplification procedure or store the extracted DNA at ≤ -18°C.

## 9. Amplification

- Every set of amplifications should include a positive and a negative control.
- Prepare for each sample, positive controls and negative control the following Master-Mix (multiply the volumes necessary for each reaction with the number **N** of reactions and add 10% more volume).
- The total volume in the final PCR reaction (with template) is **25 µl**.
- For each reaction aliquot **20 µl** of the PCR - Master Mix (each already containing primers for ***one*** specific parodontitis germ) into a sterile microtube suitable for the thermal cycler in use (as indicated in the following table):

| Preparation of PCR reagent | Reaction Volume: 20 µl | Master Mix Volume |
|---|---|---|
| **1x** Primer Mix (of ***one*** specific parodontitis germ) | 2.5 µl | 2.5 µl x N + 10 % |
| PCR Master Mix, ready to use (without primer) | 17.5 µl | 17.5 µl x N + 10 % |

- Samples: add **5 µl** of **extracted DNA** to Master Mixes prefilled with respective primers for each specific parodontitis germ; means (up to) six tubes for each individual patient sample. Do not forget the correct indication!
- Positive control: add **5 µl** of **positive control DNA** to the respective Master Mix for the positive control.
- Negative control: add **5 µl** of **negative control** to the respective Master Mix for the NTC.
- Transfer the micro tubes into the thermal cycler (if necessary overlay the Mix with 60 µl of mineral oil).
- Perform the following amplification protocol:

| | |
|---|---|
| **Initial Hold:** | **94°C** for **15 min** |
| **30 cycles:** | **94°C** for **1 min** / **61°C** for **1 min** / **72°C** for **1 min** |
| **Final Hold:** | **72°C** for **5 min**, follow up **20°C (or RT)** |

## 10. Detection of the Amplified DNA

- Carry out gel electrophoresis in **2%** agarose (or polyacrylamide 10%) for at least **100 Vh** (e. g. 80 min at 85 volt) in 1xTBE-buffer: mix about **15 - 20 µl** of each digestion mix with **4 µl** loading buffer and load the gel. Perform electrophoresis (with TBE running buffer). The length of the amplified DNA fragments can be equalized with a suitable molecular weight standard. The separated DNA is coloured by ethidium bromide or SybrGreen (5 µg/ ml) for 5 min and visualised under UV-light (312 nm).
- The PCR amplification leads for all bacteria to characteristic DNA fragments of specific length (**between 120** and **300 bp**) and for the 16S-DNA to a **489 bp** amplicon.
- The relative number of specific pathogen can be compared with the intensity of the 16S-DNA band. The presence and the quantity of each single pathogen species can be analysed.

| DNA sequence | Fragment Length (bp): |
|---|---|
| **Bacterial 16S DNA (internal control, IC)**<br>*Prevotella intermedia*<br>*Actinobacillus actinomycetemcomitans* | **489**<br>**300**<br>**220** |
| *Fusobacterium nucleatum*<br>*Porphyromonas gingivalis* | **180**<br>**161** |
| *Tannerella (Bacteroides) forsythensis* | 120 |

- In any case the negative controls must be negative for any amplification product. A very faint band of the 16S-RNA gene in the negative control can be due to the production process of TAQ-enzyme. Therefore it`s background and does not indicate a positive result for bacteria detection.
- The number of PCR cycles used generates products in exponential area of amplification, allowing for a conclusion on the density of pathogenic bacteria. An only **faint DNA band** characterises a **normal colonisation** whereas a **more distinct DNA band** indicates already **abnormal growth**. In consequence **a very strong DNA band** should be interpreted as result of a **pathologic situation** in the gingival pocket. The intensity of these DNA bands can also be measured by densitometric procedures.

## 11. Restrictions

The PCR results for all positive controls in DNA fragments of indicated length. For the samples at least the amplification of 16S-DNA fragment must be observed. If this is not the case, the samples must be tested a second time or the complete analysis must be repeated with freshly isolated DNA. If there are no positive control DNA fragments present, the amplification was incorrect and the chosen PCR conditions have to been proven/ corrected.

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EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 • FAX: 617-419-1110

www.EagleBio.com • info@eaglebio.com

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