

Extract viral DNA/RNA and bacterial DNA for detection of infectious diseases







Invitek Molecular produces the highest quality nucleic acid extraction products for infectious diseases, from research to diagnostics. Our extraction systems guarantee an excellent performance in downstream applications such as Next Generation Sequencing and multiplex PCR detection systems.

Invitek Molecular's product lines Universal and Ready-to-Prep (RTP®) for infectious diseases cover all requirements of daily routine labs. All kits guarantee reliable results and are CE certified.

## **UNIVERSAL KITS - All-rounder for the demanding diagnostic laboratory**



Isolation of viral DNA/RNA, bacterial DNA & genomic DNA



Suitable for all clinically relevant starting materials (e.g. serum, plasma, urine, blood, stool suspension, bacterial enrichment culture)



For every lab: From manual (spin column) to fully automated (magnetic beads) purification



High throughput: established protocols available for common extraction platforms



CE-certified, recommended for in-vitro diagnostic use

## Kit overview









Kit	Invisorb® Spin Universal Kit	InviMag <sup>®</sup> Universal Kit /IG	InviMag <sup>®</sup> Universal Kit / KF96	InviMag <sup>®</sup> Universal Kit / Starlet	Invisorb® Universal HTS 96 Kit/Starlet
Method	Manual	Full automation	Semi automation	Full automation	Full automation
Technology	Spin filter column	Magnetic bead	Magnetic bead	Magnetic bead	Filter plate, vacuum
Sample capacity	small to medium throughput	1-12	1- 96	1-96	1-96
Processing time	~ 30 min/sample	70 min/12 samples	70 min/96 samples	150 min/96 samples	90 min/96 samples

# Selection of pathogens and detection systems

Pathogen	Starting Material	Detection System
Viral RNA		
Norovirus	Supernatant from stool suspension	RIDA®GENE Norovirus (R-Biopharm AG, Darmstadt, Germany)
Viral DNA		
Adenovirus	Supernatant from stool suspension	RIDA®GENE Rotavirus/Adenovirus Duplex (R-Biopharm AG, Darmstadt, Germany)
human Cytomegalovirus (CMV)	Breast milk, serum, plasma, blood	CMV ELITe MGB Kit (ELITech, Puteaux, France), RealStar® CMV PCR Kit (Altona Diagnostics, Hamburg, Germany)
Ebstein-Barr-Virus (EBV)	Blood, plasma	EBV ELITe MGB Kit (ELITech, Puteaux, France), RealStar® EBV PCR Kit (Altona Diagnostics, Hamburg, Germany)
Bacterial DNA		
Chlamydia trachomatis	Urine	Cobas Taqman CT V2.0 (Roche Diagnostics, Rotkreuz, Switzerland)
Chlostridium difficile	Supernatant from stool suspension	RIDA®GENE CD Toxin A/B (R-Biopharm AG, Darmstadt, Germany)
MRSA	MRSA colony	RIDA®GENE MRSA (R-Biopharm AG, Darmstadt, Germany)
Mycobacteria tuberculosis	Buccal cells	MTB ELITe MGB Kit (ELITech, Puteaux, France)

## **READY-TO-PREP KITS - Maximum** reproducibility with manual use





Isolation of viral DNA/RNA and bacterial DNA



Suitable for all clinically relevant starting materials (e.g. serum, plasma, blood, stool suspension, bacterial enrichment culture, tissue)



Ready-to-Prep (RTP®) Technology: Pre-filled extraction tubes for one-step sample lysis, with storage of tubes at room temperature

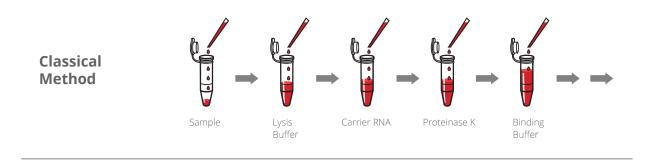


Perfect for manual use: convenient handling and maximum reproducibility



CE-certified, recommended for in-vitro diagnostic use

## Fast and convenient handling





Rapid extraction with RTP®-Technology: The extraction tube is prefilled with lyophilized buffer containing all components needed for extraction (e.g. Carrier RNA, Proteinase K). Simply add your sample and save up to 40% hands-on time compared to traditional manual extraction protocols.

# Selection of pathogens and detection systems

Pathogen	Starting Material	Downstream applications
Viral RNA		
Influenza A/B/C, H1N1, Para- influenza virus	Bronchoalveolar lavage, throat swab/nasal swab	FTD-RP33, FTD-RP21 plus (Fast-Track Diagnostics, Luxembourg) <sup>1,2,3,4</sup> , RealStar® Influenza RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) <sup>5</sup>
Corona viruses	Bronchoalveolar lavage, throat swab/nasal swab	FTD-RP33, FTD-RP21 plus (Fast-Track Diagnostics, Luxembourg) <sup>1,2,3,4</sup>
Respiratorical Syntecial Virus (RSV)	Sputum, serum, nasal swab, throat swab	FTD-RP33, FTD-RP21 plus (Fast-Track Diagnostics, Luxembourg) <sup>1,2,3,4</sup> , RSV Serotype A and B kits (Robo- screen, Leipzig, Germany) <sup>6</sup>

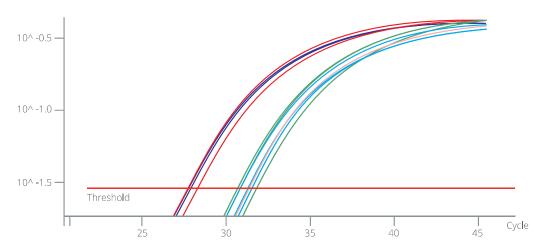
Pathogen	Starting Material	Downstream applications
Viral DNA		
Adenovirus	Water, sediments, stool suspension, sputum, serum, urine, bronchoalveolar lavage, throat swab/nasal swab	FTD-RP33, FTD-RP21 plus (Fast-Track Diagnostics, Luxembourg) <sup>1,2,3,4</sup> , Adenoplex® multiplex PCR kit (Gen-Probe Prodesse Inc., Waukesha, USA) <sup>6</sup>
Human Cytomegalovirus (CMV), Ebstein-Barr-Virus (EBV), Human Papilloma Virus (HPV)	Tissue	In-house PCR <sup>7,8,9</sup>
Herpes Simplex Virus 1 & 2, Varicella-zoster virus (VZV)	Cell culture supernatant, CSF, vesicle fluid samples	In-house PCR <sup>10,11,12,13</sup>
Bacterial DNA		
MRSA	Bacterial culture, bronchoalveolar lavage, throat swab/nasal swab	FTD-RP33 (Fast-Track Diagnostics, Luxembourg) <sup>1</sup> , EVIGENE MRSA detection kit (EVIGENE; Statens Serum Institut, Copenhagen, Denmark) <sup>14</sup>
E.coli, EHEC, EPEC	Bacterial enrichment culture	Illumina whole-genome resequencing (GATC Biotech, Eurofins Genomics, Luxembourg) <sup>15</sup> , Gene- Disc® arrays (Pall, Port Washington, USA)16
Helicobacter pylori	Bacterial enrichment culture	Pacific Biosciences RS sequencing technology (Pacific Biosciences, Menlo Park, USA) <sup>17</sup>

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# Comparison - RTP® Pathogen Kit with Invisorb® Universal Spin Kit

To compare the performance of the RTP® Pathogen Kit and Invisorb® Spin Universal Kit, RNA was prepared from 0.1  $\mu$ I and 0.01  $\mu$ I Influenza A virus stock spiked in 200  $\mu$ I serum. Three replicates each were purified with both extraction kits. PCR was performed with SureFast® Influenza A Virus Kit (Congen, Berlin, Germany) according to the manufacturer's instructions. Results show (see below) equivalent results for both extraction kits.



PCR assay SureFast® Influenza A (Congen, Berlin, Germany), FAM Channel. RNA was extracted from Influenza A spiked serum in two dilution levels with RTP® Pathogen and Invisorb® Spin Universal Kit, Blue/light blue: extraction with RTP® Pathogen. Red/light red: extraction with Invisorb® Spin Universal Kit. Green: PTC.

Ct values for samples spiked with Influenza A in two dilutions. RNA was extracted with RTP® Pathogen Kit and Invisorb® Spin Universal Kit.

RTP® Pathogen Kit			Invisor	b® Spin Unive	ersal Kit	
0.1 μl Influenza A			C	.1 µl Influenza	A	
Sample	Ct	CV [%]	Sample	Ct	CV [%]	
1	27.31	0.08	1	27.67	0.27	
2	27.23		2	27.35		
3	27.38		3	27.14		
0.01 μl Influenza A			0.01 μl Influenza A			
Sample	Ct	CV [%]	Sample	Ct	CV [%]	
Sample 1	Ct 30.96	CV [%]	Sample 1	Ct 31.09	CV [%]	
Sample  1  2			Sample 1 2			
1	30.96		1	31.09		

## **Ordering information**

Kit		Package sizes	Catalogue number
Ready-to-order			
Invisorb® Spin Universal Kit		50 preps	1050100200
	(€	250 preps	1050100300
InviMag® Universal Kit/ IG (for use on the InviGenius® PLUS, Invitek Molecular GmbH)	(€	8 x 12 preps	2450120100
InviMag® Universal Kit/ KF96 (for use on KingFisher™ 96 and KingFisher™ Flex, Thermo Fisher Scientific)	(€	5 x 96 preps	7450300200
InviMag® Universal Kit/ KF96 (without KingFisher™ plastic)	(€	5 x 96 preps	7450300250
On request			
Invisorb® Universal HTS 96 Kit/ STARlet		4 x 96 preps	7150330300
(for use on MICROLAB® STARlet, Hamilton)		24 x 96 preps	7150330400
InviMag® Universal Kit/ KFDuo w/o plastic		8 x 12 preps	2450130150
(for use on KingFisher™ Duo, Thermo Fisher Scientific)	(€	40 x 12 preps	2450130250
InviMag® Universal Kit/ STARlet (for use on MICROLAB® STARlet, Hamilton)	(€	24 x 96 preps	7450330400

Kit		Package sizes	Catalogue number
Membrane adsorption			
RTP® Pathogen Kit		50 preps	1040500200
	(€	250 preps	1040500300
Automated using magnetic be	ads		
InviMag® Pathogen Kit/ KF96 (for use on KingFisher™ 96 and KingFisher™ Fisher Scientific)	flex, Thermo	5 x 96 preps	7444050200







Compliance with EU Directive 98/79/EC on in vitro medical devices. Not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

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Invitek Molecular GmbH Robert-Rössle-Str. 10 13125 Berlin Germany

Phone: +49 30 9489 2908 info@invitek-molecular.com www.invitek-molecular.com



Collect, stabilize & extract DNA for gut microbiome profile studies





# Sample Collection Tubes with DNA Stabilizer







Transport and store stabilized DNA at ambient temperature for 3 months – no cold chain required

Eliminate the costs associated with temperature-controlled shipping

Minimize bias introduced by microbial growth and DNA degradation

Product name	Package size	Catalogue number
Stool DNA Stabilizer	180 ml	1038111100
Stool Collection Tubes with DNA Stabilizer	50 tubes	1038111200
	250 tubes	1038111300

## **Extraction kits**

Invitek Molecular is committed to developing products for state-of-the-art sample collection and nucleic acid extraction used in microbiome research. Maintaining the integrity of samples collected is a major challenge. Our collection and extraction systems guarantee an excellent performance in NGS downstream applications.





Remove all PCR inhibitors from DNA samples



Isolate DNA from microorganisms or from the host organism



Extract nucleic acids from food and feed residues of plant or animal origin from the stool sample

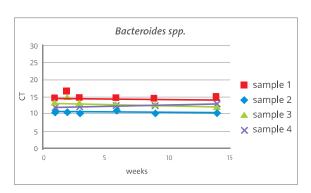


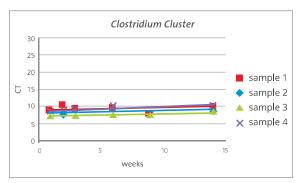
Obtain high quality DNA suitable for 16S rRNA Microbiome profiling, Shotgun Metagenomic Sequencing, qPCR and arrays

### Fast and convenient handling

The kits contain the InviAdsorb matrix in order to remove all effective inhibitors very efficiently. After a rigorous pre-lysis step using Zirconia Beads with optimized pre-lysis buffer at high temperatures, the sample is pre-incubated with InviAdsorb to remove PCR inhibitors. Proteinase K digestion ensures high yields also from Gram-positive bacteria. Stool samples contain a range of DNA e.g. host DNA from colon epithelial cells, parasite DNA, bacterial DNA, DNA from food or DNA from gastrointestinal pathogens. The choice of different lysis conditions enables the accumulation or reduction of the bacterial DNA content in the total DNA.

### DNA extraction after sample storage at ambient temperatures





1.4 ml of preserved stool samples were tested on day 1, day 2, day 7, day 18, day 49 and day 105 for DNA isolation using the manual spin kit. 2  $\mu$ l eluate were used in the RIDA®GENE Gut Balance real-time PCR assay from R-Biopharm (Darmstadt, Germany).

Kit	Starting material	Package sizes	Catalogue number
Membrane adsorption			
PSP® Spin Stool DNA Basic Kit <b>(€</b> (without lysis buffer)*	1.4 ml Stool DNA Stabilizer with stool homogenate	50 preps 250 preps	1038120200 1038120300
Automated using magnetic beads			
InviMag® Stool DNA Kit/KF96 (for use on KingFisher™ 96 and KingFisher™ Flex, Thermo Fisher Scientific)	up to 200 - 400 mg	5x96 preps	7438300200

<sup>\*)</sup> For sample lysis, the kit needs to be combined with Stool Collection Tubes with Stool DNA Stabilizer or Stool DNA Stabilizer (180 ml for 50 preps, 2 x 180 ml for 250 preps), for ordering information please refer to page 2.







Compliance with EU Directive 98/79/EC on in vitro medical devices. Not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

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Invitek Molecular GmbH

Robert-Rössle-Str. 10 13125 Berlin Germany

Phone: +49 30 9489 2908 info@invitek-molecular.com www.invitek-molecular.com

## Invitek Molecular products for reliable Microbiome studies

The effect of human gut microbiota on the health and pathogenesis of various diseases have been investigated in recent years. Changes in the microbiome or the presence of pathogenic microbes can cause diseases such as obesity, diabetes, allergies, cancer etc. Analyzing the human microbiota plays a major role in personalized medicine or dietary recommendations due to the presence of enormous microbial diversity among individuals. High-throughput sequencing technology, coupled with the use of conserved marker genes has allowed for the understanding of microbial communities.

A major challenge for microbiome studies is maintaining an even and accurate DNA extraction in the presence of samples with a wide range of bacterial content. Therefore, sample stabilization and validated DNA extraction are essential steps in gut microbiome analysis projects. Severe errors and biases are caused by sample storage and extraction procedures. Especially the DNA isolation procedure continues to be a large source of experimental variability in 16S rRNA sequence analysis and the most time-consuming step in a high-throughput pipeline. Here, we review the use of Invitek Molecular products for stool sample stabilization and DNA extraction for microbiome studies and several different applications. The most common downstream application was the NGS sequencing on the Illumina MiSeq platform, targeting the V3–V4 region of the 16S rRNA genes.

## The Invitek Molecular Microbiome products

Invitek Molecular offer products for stool sample collection and stabilization as well as DNA extraction kits for manual and automated use.

#### Collection modules

The Stool Collection Tube is designed for stool sample collection and stabilization using a liquid stabilization buffer, which stabilizies the total DNA for three months at room temperature. The DNA Stabilizer effects inactivation of DNases and prevents degradation of DNA. Furthermore, it preserves the microorganism titer and pre-lyses bacteria. The Collection Tube features an integrated spoon in the lid for sampling and a label for donor description. Each tube is packed separately in a plastic bag, including an instruction leaflet in several languages.

#### **Extraction kits**

Invitek Molecular offers several ready-to-use kits for DNA extraction from stool samples: The PSP Spin Stool DNA Kit enables a fast and easy purification of total DNA from max. 200 mg of fresh or frozen stool samples using a spin column-based procedure. The PSP Spin Stool DNA Plus Kit is an integrated system for collection, transportation and storage of stool samples using the Stool Collection Tubes and subsequent DNA purification using spin columns. The PSP Spin Stool DNA Basic Kit is a DNA purification system from stool samples in combination with the corresponding Stool Collection Tubes (extra module, recommended when to use separately). For automated use, the InviMag Stool DNA Kits enable an efficient high-throughput isolation of total DNA from up to 200–400 mg of stool samples using magnetic beads on the KingFisher workstations (Thermo Scientific).

The kits were developed to isolate DNA from pathogenic microorganisms and from the host organism. Furthermore, it is possible to extract nucleic acids from food and feed residues of plant or animal origin from the stool sample. The kits are CE-marked and recommended for in-vitro diagnostic use.



### Extraction procedure

Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. To ensure removal of these contaminants, the Stool DNA kits contain the InviAdsorb matrix in order to remove all effective inhibitors very efficiently. After a rigorous pre-lysis step using Zirconia Beads with optimized pre-lysis buffer at high temperatures, the sample is pre-incubated with InviAdsorb to remove PCR inhibitors. Undissolved particles and PCR inhibitors bound to InviAdsorb are removed by a centrifugation step. The following Proteinase K digestion ensures high yields also from Gram-positive bacteria. Stool samples contain a range of DNA e.g. host DNA from colon epithelial cells, parasite DNA, bacterial DNA, DNA from food or DNA from gastrointestinal pathogens. The choice of different lysis conditions enables the accumulation or reduction of the bacterial DNA content in the total DNA. All impurities are removed very efficiently during the washing steps and the purified DNA is eluted directly in a low-salt buffer.

## **Applications**

For many years, the Invitek Molecular products have been used in several microbiome studies with different focuses. Due to the complexity of the starting material (DNA content, impurities, inhibitors) and variations caused by sample storage and extraction procedures, the fecal sample processing must be optimized according to the microbiome study. Several studies and reviews compare different commercially available extraction kits, sampling, stabilization and storage procedures [1,2,3]. Wu et al. [4] showed that the Stool DNA Stabilizer from the PSP Spin Stool DNA Plus Kit was able to sustain gut microbiota profile effectively for up to 48 hours.

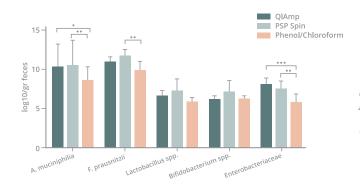
extraction kits 50 preps/kit)	PowerSoil® DNA isolation Kitb	QlAmp® DNA Stool Mini Kit	FastDNA™ SPIN Kit for Feces	PSP® Spin Stool DNA Kit	QLAmp® UCP Mini Kit <sup>9</sup>
Recommended fecal weight	250 mg	220 mg	500 mg	200 mg	NA
Bead tubes	✓ (Ceramic)	Х	✓ (Mixed)	✓ (Zirconia)	Х
Popularity	<b>√√√</b>	<b>√√</b> √	√√	√√	✓
Estimaded price <sup>a</sup> (USD)	\$ 279.00	\$ 234.00	\$ 306.15	NA	\$ 315.00
Others	used in HMP	fast and easy use	multiple size beads	NA	for low biomass

<sup>&</sup>lt;sup>a</sup> Price information are acquired from official website of manufactures.

[1] Optimization of fecal sample processing for microbiome study - The journey from bathroom to bench.

Wu WK, Chen CC, Panyod S, Chen RA, Wu MS, Sheen LY, Chang SC. J Formos Med Assoc. 2018 Feb 25. pii: S0929-6646(17)30857-4.

Amplicon sequencing requires the extraction of DNA from a diverse and complex mixture of microorganisms whose identity is determined at the end of the experiment. Therefore, DNA extraction needs to be effective for a wide range of species; efficient extraction is crucial and its influence on the outcome is significant. Özkul showed that the PSP Spin Stool Kit resulted in a higher DNA purity and higher yield from different species compared to other methods [5].



[5] Comparison of Bacterial DNA Extraction Methods From Stool Samples for Quantitative Real-Time PCR Analysis. C. Özkul / Hacettepe J. Biol. & Chem., 2020, 48 (1), 41-48

Comparison of log10/gram feces levels of bacterial groups between different extraction methods. Multiple comparisons were performed by two-way ANOVA. \*p<0.05; \*\*p<0.01; \*\*\*p<0.011



<sup>&</sup>lt;sup>b</sup> The kit of PowerSoil is renamed as PowerFecal with identical kit contents except the bead tubes are not prefilled with bead solution according to the distributer. NA, not available

#### Microbiome associated diseases

Moreover, studies show an association between the human microbiota and several diseases (see table 1). Diagnostic applications of the microbiome can be divided into two categories: diagnosis of infectious diseases and monitoring of microbial components of chronic diseases.

Chronic diseases	Reference
Diabetes	6,7,8,9
Obesity	10, 11, 12
Cancer (Colon, stomach)	13, 14, 15
Parkinson	16, 17
Fibromyalgia	18
Coronary heart disease	19
HIV infection	20, 21, 22
Inflammatory Bowel Disease	23

Infectious diseases	Reference
Helicobacter pylori	24
Clostridium difficile	25, 26
Pseudomonas aeruginosa	27
Enterocytozoon bieneusi	28, 29
Cryptosporidium	30

Table 1: Overview of peer-reviewed publications

For example, Boertien et al. [17] showed that the PSP Spin Stool DNA Plus Kit was used in several Microbiome studies in Parkinsons´s disease. In this review, the authors highlighted that sampling procedures can introduce bias during sampling itself or during transportation and storage. Shipping time was identified as a significant contributor to microbiome composition changes. Inadequate adjustment for microbial shifts due to sample transportation conditions can therefore be a potential source for both intra- and inter-study differences.

### **Nutrigenomics**

Nutrigenomics is the study of the effects of food and food constituents on gene expression, and how genetic variations affect the nutritional environment. It focuses on understanding the interaction between nutrients and other dietary bioactives with the genome at the molecular level, to understand how specific nutrients or dietary regimes may affect human health. The PSP Spin Stool Kits were used in studies where a Microbiome-dependent relationship between diet and metabolites was shown [31, 32, 33] and how breast milk feeding influences the infant microbiome [34, 35].

### Microbiome in animals

With the advances of new technologies, it is now possible to entangle complex microbial communities found across animal kingdoms. In several studies, the animal microbiome in livestock, domestic pets, and wild animals was analyzed using the Invitek Molecular Stool Kits and the Stool Collection Tubes (see table 2). For example, mice are often used as animal models for microbiome research whereas in wild animals or domestic pets, infectious diseases or parasites were detected.



Animal	Reference
Mice	36, 37, 38
Reindeer	39
Cows (milk, rumen)	40, 41, 42, 43
Goats	44
Foxes	45, 46
Bears	47
Birds	48, 49
Dogs	46, 49
Sloths	50
Chimpanzees and gorillas	51
Pandas	52

Table 2: Overview of peer-reviewed publications

### Fungal DNA in stool samples

Several fungal DNA extraction methods have recently been evaluated for the study of the intestinal mycobiome. As for prokaryotic cells, the main challenge during the extraction of DNA from fungi is the lysis of the cell wall. An inclusion of an extra initial bead beating step when using the PSP Spin Stool DNA Plus Kit can improve the extraction of fungal DNA [53].

### **Conclusion**

Invitek Molecular has offered complete solutions for reliable and validated microbiome sample management, which has been shown in many peer-reviewed publications

- Microbial & host DNA preserved at room temperature in the pre-filled buffer for 3 months at room temperature
- Collection and stabilization of DNA in the Stool Collection Tubes
- Extraction of total DNA with spin-column kits or automated kits using magnetic beads in 96-well format
- Removal of PCR inhibitors from DNA samples
- Isolation of high-quality total DNA for PCR and NGS applications (Microbiome/Metagenomic sequencing)

### References

- Optimization of fecal sample processing for microbiome study The journey from bathroom to bench. Wu WK, Chen CC, Panyod S, Chen RA, Wu MS, Sheen LY, Chang SC. J Formos Med Assoc. 2018 Feb 25. pii: S0929-6646(17)30857-4
- The effect of storage conditions on microbial communities in stool. Nel Van Zyl K, Whitelaw AC, Newton-Foot M1. PLoS One. 2020 Jan 14;15(1):e0227486
- Interpersonal Variations in Gut Microbiota Profiles Supersedes the Effects of Differing Fecal Storage Conditions.

  Bundgaard-Nielsen C, Hagstrøm S, Sørensen S. Sci Rep. 2018 Nov 26;8(1):17367
- Wu, G. D. et al. Sampling and pyrosequencing methods for characterizing bacterial communities in the human gut using 16S sequence tags. BMC Microbiol. 10, 206 (2010)
- Comparison of Bacterial DNA Extraction Methods From Stool Samples for Quantitative Real-Time PCR Analysis.

  C. Özkul / Hacettepe J. Biol. & Chem., 2020, 48 (1), 41-48
- Salguero MV, Al-Obaide MAI, Singh R, Siepmann T, Vasylyeva TL. Dysbiosis of Gram-negative gut microbiota and the asso ciated serum lipopolysaccharide exacerbates inflammation in type 2 diabetic patients with chronic kidney disease. Exp Ther Med. 2019;18(5):3461-3469



- Gut microbiome in gestational diabetes: a cross-sectional study of mothers and offspring 5 years postpartum.

  Hasan S, Aho V, Pereira P, Paulin L, Koivusalo SB, Auvinen P, Eriksson JG. Acta Obstet Gynecol Scand. 2018 Jan;97(1):38-46.
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