

(READY-TO-PREP) RTP® Pathogen Kit for manual DNA/RNA extraction from pathogens

One of the greatest public health challenges of the 21st century are infectious diseases, due to complex and interrelated factors such as population growth, climate change, and geopolitics. Accurate diagnostic tools are needed for early detection of infectious diseases to prevent disease outbreaks. These diagnostic tools provide vital information for epidemiological models, disease tracking, treatment selection, and epidemic prevention. Diagnosis of infectious diseases at the genomic level using nucleic acid biomarkers has proven to be the most effective approach to date.

A nucleic acid extraction method specific to the sample material is of great importance for the success and informative value of sensitive downstream methods, such as amplification techniques. In human diagnostics, a wide variety of sample matrices are tested for pathogens and infectious diseases, e.g., swabs, tissue, stool samples or body fluids. Following sample extraction, various PCR techniques are used to detect a variety of pathogens of viral and bacterial origin. A particularly popular tool for straightforward sample analysis is multiplex PCR, which can detect viral DNA and RNA as well as bacterial DNA in a single assay. This method is frequently used for the diagnosis of respiratory infections. For the diverse sample matrices of human diagnostic and sophisticated downstream assays, the RTP® Pathogen Kit is the ideal application for sample extraction. Different nucleic acid types of viral and bacterial origin are extracted simultaneously. In addition, the RTP® Pathogen Kit offers a unique 1-step lysis, which increases reproducibility and helps to minimize pipetting steps in manual sample extraction.

RTP® - Ready-To-Prep

The RTP® Pathogen Kit greatly simplifies DNA/RNA purification from pathogen-containing samples. Everything needed for DNA/RNA sample preparation - lysis buffer, Proteinase K, lysozyme and carrier nucleic acids - is provided in a lyophilized format in an Extraction Tube (Fig. 1). The lyophilized buffer is ready-to-use and stable at room temperature. The kit is CE-IVD certified and complies with REGULATION (EU) 2017/746 on *in vitro* diagnostic medical devices.



Extraction Tube with lyophilized components

- ✓ Lysis Buffer
- ✓ Proteinase K / Lysozyme
- ✓ Carrier nucleic acids
- ✓ stable at RT

Figure 1: The Extraction Tube of the RTP® Pathogen Kit contains all components for sample lysis in a lyophilized buffer.

EXTRACTION PROCEDURE

The one-step sample lysis takes place under non-chaotropic conditions, this gentle sample treatment allows potential inhibitors in the sample to be removed while nucleic acids remain intact. Due to the carrier nucleic acids in the extraction buffer, the recovery of small amounts of target sequences is enhanced.

After heat induced lysis, samples are transferred to a spin column-based procedure with a total preparation time of only 20 minutes. Impurities are removed by repeated washing steps, finally the purified nucleic acids can be eluted in a small volume of elution buffer.



Fig. 2: Invitek extraction kit

FAST AND CONVENIENT HANDLING

The RTP® technology reduces hands-on time and plastic waste while increasing reproducibility and safety. Up to 60% fewer pipetting steps and pipet tip consumption can be achieved with the one-step lysis compared to conventional kits (Fig. 3). Due to the broad-spectrum specification of the RTP® Pathogen Kit in terms of sample material and target nucleic acid, the kit can be used ubiquitously in the laboratory and enables a wide range of applications.

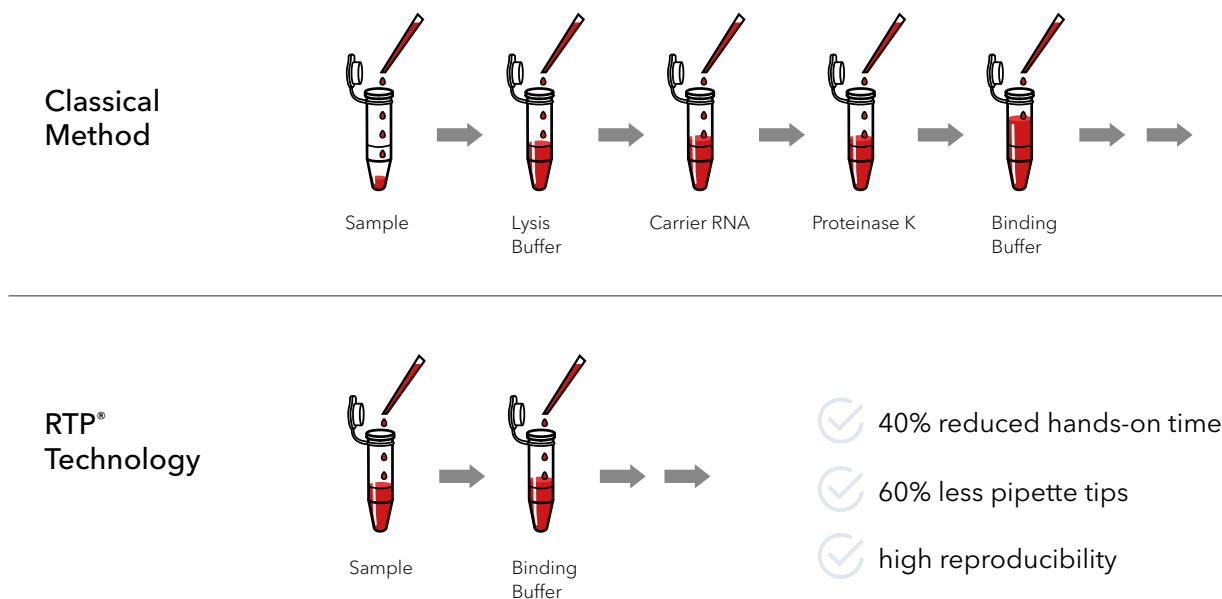


Figure 3: Comparison of a classical spin column extraction method with the RTP® technology. Due to a 1-step lysis, hands-on time and pipetting steps are reduced significantly during lysis while ensuring highest reproducibility.

Applications using the RTP® kit

For many years, Invitek’s products have been used for the detection of infectious diseases, e.g., respiratory RNA viruses, DNA viruses such as adenovirus or herpes and various bacterial species. Due to the high-quality nucleic acids obtained with the extraction protocol classical and advanced molecular biological methods can be applied like multiplex qPCR and qRT-PCR and Next-Generation-Sequencing. Apart from diagnostic assays, the RTP® kit already has been used for diverse microbiome profiling studies.

1. DNA extraction from bacteria

The literature encompasses diverse applications for isolating bacterial DNA, such as identifying human diseases, conducting genetic analyses of bacterial species, and detecting bacteria in food and environmental samples.

Table 1: Applications of the RTP® Pathogen Kit for bacterial detection in diagnostics and research

Pathogen	Starting material	Detection system
<i>E.coli</i> , EHEC, EPEC	Bacterial enrichment culture	Illumina whole-genome resequencing (GATC Biotech, Eurofins Genomics, Luxembourg) ¹ , GeneDisc® arrays (Pall, Port Washington, USA) ²
<i>Helicobacter pylori</i>	Bacterial enrichment culture	Pacific Biosciences RS sequencing technology (Pacific Biosciences, Menlo Park, USA) ³
<i>Legionella pneumophila</i>	Water, lung tissue or sputum	in-house PCR ^{4,5}
<i>Mycoplasma gallisepticum</i> , <i>Mycoplasma synoviae</i> , <i>Mycoplasma pneumoniae</i>	Bacterial culture, oropharyngeal swabs	in-house PCR ^{6,7,8}
<i>Yersinia enterocolitica</i>	Bacterial culture	in-house PCR ⁹
<i>Pseudomonas aeruginosa</i>	Sputum	in-house PCR ¹⁰
<i>Salmonella enterica</i>	Bacterial culture	in-house PCR, microarray, multilocus sequence typing ^{11,12}
<i>Vibrio cholerae</i> , <i>Vibrio vulnificus</i> , <i>Vibrio parahaemolyticus</i> , other <i>Vibrionaceae</i>	Bacterial culture	in-house PCR ^{13,14,15,16,17}
<i>Treponema spp.</i>	Serum and CSF samples, brain tissue	in-house PCR ¹⁸
MRSA	Bacterial culture, bronchoalveolar lavage, throat swab/nasal swab	FTD-RP33 (Fast-Track Diagnostics, Luxembourg) ¹⁹ , EVIGENE MRSA detection kit (EVIGENE; Statens Serum Institut, Copenhagen, Denmark) ²⁰
<i>Bacillus cereus</i>	Bacterial culture	in-house PCR ²¹
<i>Mycobacterium tuberculosis</i>	Sputum	Genotype-MTBDR assay (Hain Life-science, Nehren, Germany) ²²
<i>Chlamydia trachomatis</i>	Urogenital swabs	in-house PCR ²³
<i>Periodontal bacteria (Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Treponema denticola, Prevotella intermedia)</i>	Paper points, saliva samples, plaque samples	in-house PCR ²⁴ , micro-IDent microorganism kit Hain Lifescience GmbH, Nehren, Germany ²⁵
<i>Environmental bacteria (red sea bacterial strains)</i>	Bacterial culture	in-house PCR ²⁶

Furthermore, the RTP® Pathogen Kit was used for microbiome analysis in human and plant samples:

Table 2: Applications of the RTP® Pathogen Kit for microbiome analysis

Target microbiome	Starting material	Downstream application
Gut	Bacterial culture	in-house PCR ^{27,28}
Skin	Skin swabs	in-house PCR ²⁹
Vaginal	Vaginal swabs	Next Generation Sequencing ³⁰
Oral	Paper points, saliva, oral biofilms from membrane filters	Pyrosequencing ³¹ , sequencing ³²
Plant	Potato, carrot, beet, neep, topinambur	in-house PCR ³³

2. DNA/RNA extraction from viruses

Here, we present a listing of literature wherein viral RNA and DNA isolation is documented for the purpose of detecting viral diseases, including respiratory or tropical diseases.

Table 3: Applications of the RTP® Pathogen Kit for viral detection in diagnostics and research

Pathogen	Starting material	Detection system
Viral RNA		
<i>SARS-CoV-2 and other Corona viruses</i>	Nasopharyngeal swab, bronchoalveolar lavage	in-house PCR ^{34,35} , FTD-RP33, FTD-RP21 plus (Fast-Track Diagnostics, Luxembourg) ^{19,36,37,38}
<i>Influenza A/B/C, H1N1, Parainfluenza virus</i>	Nasopharyngeal swab, bronchoalveolar lavage	FTD-RP33, FTD-RP21 plus (Fast-Track Diagnostics, Luxembourg) ^{19,36,37,38} , RealStar® Influenza RT PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) ³⁹
<i>Respiratory syncytial virus (RSV)</i>	Sputum, serum, nasal swab, throat swab	FTD-RP33, FTD-RP21 plus (Fast-Track Diagnostics, Luxembourg) ^{19,36,37,38} , RSV Serotype A and B kits (Roboscreen, Leipzig, Germany) ⁴⁰
<i>Zika virus</i>	Serum, urine samples	in-house PCR ⁴¹
<i>Crimean-Congo hemorrhagic fever virus</i>	Ticks	in-house PCR, RealStar CCHFV RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) ⁴²
<i>Rotavirus</i>	Virus culture	in-house PCR ⁴³
<i>Usutu virus</i>	Mosquitoes, tissue samples	in-house PCR ⁴⁴
<i>Phlebovirus and Massilia virus</i>	Sandflies	in-house PCR ⁴⁵
<i>Tick-borne encephalitis virus (TBEV)</i>	Tissue sample (spleen)	in-house PCR ⁴⁶
<i>Hepatitis C, E</i>	Liver tissue	INNO-LiPA™ HCV II kit (Innogenetics, Ghent, Belgium) ⁴⁷ , in-house PCR ⁴⁸
Viral DNA		
<i>Adenovirus</i>	Water, sediments, stool suspension, sputum, serum, urine, bronchoalveolar lavage, throat swab/nasal swab	FTD-RP33, FTD-RP21 plus (Fast-Track Diagnostics, Luxembourg) ^{19,36,37,38} , Adenoplex® multiplex PCR kit (Gen-Probe Prodesse Inc., Waukesha, USA) ⁴⁰
<i>Human Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Papilloma Virus (HPV)</i>	Tissue	in-house PCR ^{49,50,51}
<i>Herpes Simplex Virus 1 & 2, Varicella-zoster virus (VZV)</i>	Cell culture supernatant, CSF, vesicle fluid samples	in-house PCR ^{52,53,54,55}
<i>Hepatitis B</i>	Oral fluid samples	in-house PCR, Abbott Real Time HBV (Abbott, Abbott Park, Illinois, USA) ⁵⁶
<i>Equid herpesviruses</i>	Cell culture supernatant, swabs	in-house PCR ⁵⁷
<i>African swine fever virus</i>	Insect tissue	in-house PCR ⁵⁸
<i>BK and JC polyomaviruses</i>	Tissue	in-house PCR ⁵⁹
<i>Orthopoxvirus</i>	Blotting paper	in-house PCR ⁶⁰

RTP® Pathogen Kit in comparison to other target specific extraction kits

Previously, there were several RTP® kits specifically offered for bacteria or viruses (RTP® Bacteria DNA Mini Kit, RTP® DNA/RNA Virus Mini Kit, RTP® Mycobacteria Kit). For easier and more flexible handling, Invitek offers the RTP® Pathogen Kit with different protocols for bacteria and viruses combined in one kit.

To compare the performance of the RTP® Pathogen Kit and RTP® DNA/RNA Virus Mini Kit, RNA was prepared from 0.1 µl Influenza A virus stock spiked in 200 µl 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 (Fig.4) equivalent results for both extraction kits. Additionally, to compare the performance of the RTP® Pathogen Kit and RTP® Bacteria DNA Mini Kit, three replicates each were extracted from a *Bacillus subtilis* overnight culture. In the subsequent kit performance assay, PCR was done specifically for the internal control: ECD = Extraction Control DNA. Results show (Fig.5) equivalent results for both extraction kits.

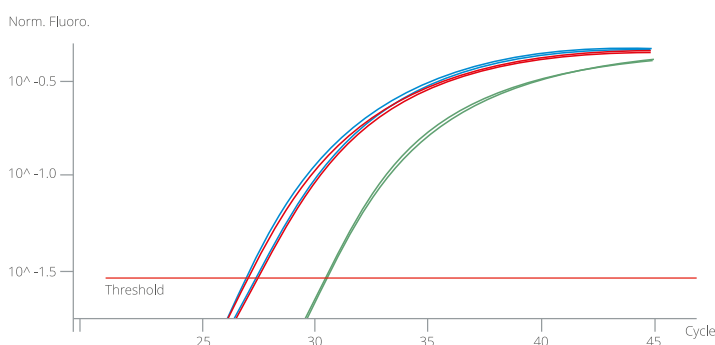


Figure 4: PCR assay SureFast® Influenza A (Congen, Berlin, Germany), FAM Channel. RNA was extracted from Influenza A spiked serum with RTP® Pathogen Kit and RTP® DNA/RNA Virus Mini Kit. Blue: extraction with RTP® Pathogen Kit. Red: extraction with RTP® DNA/RNA Virus Mini Kit. Green: PTC.

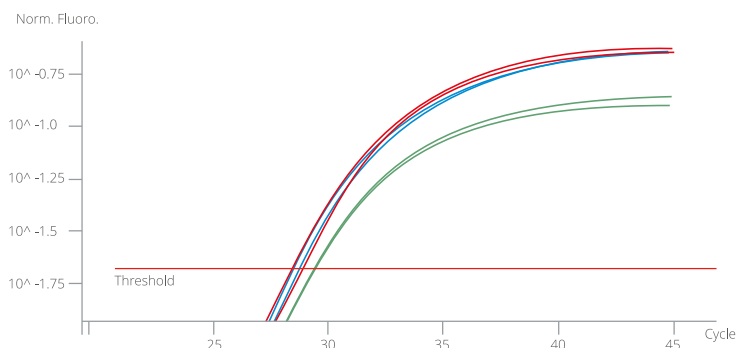


Figure 5: PCR assay specific for ECD, FAM Channel. Blue: extraction with RTP® Pathogen Kit. Red: extraction with RTP® Bacteria DNA Mini Kit. Green: PTC.

Conclusion

Invitek Diagnostics offers a reliable and convenient solution for nucleic acid extraction from bacteria, and viruses, which has been shown in many peer-reviewed publications.

- Isolation of viral DNA/RNA and bacterial DNA
- Suitable for all clinically relevant starting materials (e.g. serum, plasma, stool suspension, bacterial enrichment culture, tissue)
- Ready-to-Prep (RTP®) Technology: Pre-filled Extraction Tubes for one-step sample lysis, kits can be stored at room temperature
- Perfect for manual use: convenient handling and maximum reproducibility
- CE-IVD certified, recommended for in vitro diagnostic use

For automated use, we recommend the Invitek Universal Kit series which can be easily automated on several automated platforms, such as the KingFisher™ Flex.

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