September 2022 | EN

virotype® Influenza A 2.0 RT-PCR Kit Handbook

For detection of RNA from Influenza A Virus

Licensed in accordance with § 11 (2) of the German Animal Health Act
MA No.: FLI-C 116

REF 100 reactions (Cat. no. VT282625)

INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

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Location: Moldova

INDICAL BIOSCIENCE

Contents

Kit contents

virotype Influenza A 2.0 RT-PCR Kit **Cat. no.** **Number of reactions**	**(100)** **VT282625** **100**
Master Mix (tube with orange cap) includes enzymes, primers, and probes	2 x 1000 µl
Positive Control (tube with red cap)	1 x 150 µl
Negative Control (tube with blue cap)	1 x 150 µl
Handbook	1

Intended use

The virotype Influenza A 2.0 RT-PCR Kit is intended for the detection of RNA from Influenza A Virus in oropharyngeal, tracheal, and cloacal swabs (individual or pooled), fecal samples, or tissue samples from birds; nasal swabs, bronchoalveolar lavage fluid (BALF), and tissue samples from swine, as well as nasal swabs from equids.

The kit is approved by the Friedrich-Loeffler-Institut and licensed in accordance with § 11 (2) of the German Animal Health Act (FLI-C 116) for use in Germany for veterinary diagnostic procedures.

For veterinary use only.

Symbols

	Legal manufacturer
	Lot number
	Use by date
	Temperature limitations for storage
	Handbook
	Catalog number
	Material number
	Protect from light
	For samples from birds, swine, and equids

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of virotype Influenza A 2.0 RT-PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the virotype Influenza A 2.0 RT-PCR Kit should be stored at -30°C to -15°C and are stable until the expiration date stated on the label. Avoid repeated thawing and freezing (> 3x), as this may reduce assay sensitivity. Freeze the components in aliquots if they will only be used intermittently.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available from your local sales representative or by Email request under **compliance@indical.com**.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Introduction

The virotype Influenza A 2.0 RT-PCR Kit is a highly sensitive solution for the safe and sensitive detection of RNA from Influenza A Virus in samples from birds, pigs, and equids.

The Influenza A Virus is an enveloped, single-stranded RNA virus and belongs to the family *Orthomyxoviridae*, genus *Alphainfluenzavirus*. Influenza A viruses occur in high genetic diversity and a wide range of virulence. They are grouped into low and highly pathogenic strains. Waterfowl are the natural reservoir of Low Pathogenic Avian Influenza Viruses (LPAIV). Highly Pathogenic Avian Influenza Viruses (HPAIV) belong to subtypes H5 or H7 and may cause fowl plague in domestic poultry with high economic losses.

The subtypes H1N1, H1N2, and H3N2 of Influenza A Virus can also cause infections of the respiratory tract in swine as can the subtype H3N8 in equids.

Principle

Polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is identified using fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i.e., in real-time) allows detection of the accumulating product without the need to re-open the reaction tubes afterward.

The virotype Influenza A 2.0 RT-PCR Kit contains all of the necessary reagents for the detection of Influenza A Virus RNA, including a Positive and Negative Control. With this kit, both, reverse transcription and PCR are performed in one reaction tube, reducing the risk of contamination.

The virotype Influenza A 2.0 RT-PCR Kit uses two specific primer/probe combinations:

- FAM™ fluorescence for RNA of Influenza A Virus
- HEX™ fluorescence for the endogenous Internal Control (EC; β-actin present within the sample)

A Positive Control serves to verify the functionality of the reaction mix for the amplification of the Influenza A Virus RNA target.

RNA extraction

The virotype Influenza A 2.0 RT-PCR Kit can be used for the detection of Influenza A Virus RNA from the following sample types:

- Birds: oropharyngeal, tracheal, and cloacal swabs (individual or pooled), fecal samples, tissue samples
- Swine: nasal swabs, bronchoalveolar lavage fluid (BALF), tissue samples
- Equids: nasal swabs

Due to the high sensitivity of the test, pools of up to 10 individual swab samples can be tested.

Note: For use in Germany the specifications described in the „*Amtliche Methodensammlung*“ apply.

Prior to real-time RT-PCR, viral RNA must be extracted from the starting material.

INDICAL offers a range of validated kits for the extraction of RNA from animal samples.

Extraction based on magnetic beads:

- **IndiMag® Pathogen Kit** (SP947457)
- **IndiMag Pathogen Kitw/o plastics** (SP947257)
- **IndiMag Pathogen IM48 Cartridge** (SP947654P608, SP947654P224)
- **IndiMag Pathogen KF96 Cartridge** (SP947855P196)

Extraction based on spin columns:

- **IndiSpin® Pathogen Kit** (SP54104, SP54106)
- **IndiSpin QIAcube® HT Pathogen Kit** (SP54161)

If real-time RT-PCR is not performed immediately after extraction, store the RNA at -20°C or at -80°C for longer storage.

For further information on automated and manual extraction of Influenza A Virus RNA from different sample types, refer to the respective handbook or contact INDICAL Support at **support@indical.com**.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Pipets
- Nuclease-free, aerosol-resistant pipet tips with filters
- Sterile 1.5 ml Eppendorf® tubes
- Nuclease-free (RNase/ DNase-free) consumables. Special care should be taken to avoid nuclease contamination of all reagents and consumables used to set up PCR for sensitive identification of viral nucleic acids
- Cooling device or ice
- Benchtop centrifuge with rotor for 1.5 ml tubes
- Real-time cycler with appropriate fluorescent channels
- Appropriate software for chosen real-time cycler
- Appropriate strip tubes and caps or 96-well optical microplate with optical sealing film or cover for chosen real-time cycler

Important notes

General precautions

The user should always pay attention to the following:

- Use nuclease-free pipet tips with filters.
- Store and extract positive materials (specimens, positive controls and amplicons) separately from all other reagents and add them to the reaction mix in a spatially separated facility.
- Thaw all components on ice before starting the assay.
- When thawed, mix the components by inverting and centrifuge briefly.
- Do not use components of the test kit past the expiration date.
- Keep samples and controls on ice or in a cooling block during the setup of reactions.

Negative control

At least one negative control reaction should be included in each PCR run, containing all the components of the reaction except for the pathogen template. This enables assessment of contamination in the reaction.

Positive control

When performing PCR on unknown samples, it is recommended to perform a positive control reaction in the PCR run, containing a sample that is known to include the targeted viral RNA. A positive control serves to prove the functionality of the pathogen assay, e.g., the correct setup of the reaction mix. Use 5 µl of the Positive Control provided with

the virotype Influenza A 2.0 RT-PCR Kit to test for successful amplification of the target.

Extraction and amplification control

For increased process safety and convenience, one extraction and amplification control assay is included in the test kit.

An endogenous Internal Control (EC) detects the β-actin gene present within the sample. This allows extraction and amplification to be monitored.

Protocol: Real-time RT-PCR for detection of RNA from Influenza A Virus

Important points before starting

- Please read „Important notes“ on page 10 before starting.
- Include at least one positive control (Positive Control) and one negative control (Negative Control) per PCR run.
- Before beginning the procedure, read through the protocol and ensure that you are familiar with the operation of the chosen real-time PCR cycler.
- RNA is unstable. Perform the protocol without interruption.

Things to do before starting

- Thaw all reagents on ice and protect from light.
- Before use, spin the reagents briefly.
- Maintain reagents on ice or in a cooling block during PCR setup.

Procedure

1. Before use, mix the Master Mix by inverting 5 times or until mixed thoroughly, then centrifuge briefly to collect the fluids.
2. Pipet 20 µl of the Master Mix into each reaction tube. Then add 5 µl of the sample RNA (Table 1).

   Include positive and negative control reactions.

   Positive Control: Use 5 µl of the positive control (Positive Control) instead of sample RNA.

   Negative Control: Use 5 µl of the negative control (Negative Control) instead of sample RNA.

Table 1. Preparation of reaction mix

Component	**Volume**
Master Mix	20 µl
Sample	5 µl
Total volume	**25 µl**

3. Close the reaction tubes or seal the plate and invert 5 times or until mixed thoroughly. Then centrifuge briefly to collect the fluids.

4. Set the filters for the reporter dyes in the software of your thermal cycler according to Table 2.

Table 2. Filter settings for the reporter

Pathogen/ Internal Control	Reporter
Influenza A Virus	FAM
Endogenous Internal Control (EC)	HEX™[1]
Passive reference[2]	ROX™

1 Use the option appropriate for your thermal cycler.
2 Internal reference for use with ABI PRISM® Sequence Detection Systems (Applied Biosystems®)

5. Run the real-time PCR protocol according to Table 3.

Table 3. Real-time RT-PCR protocol for Influenza A 2.0

Step	Temperature	Time	Number of cycles
Reverse Transcription	50°C	10 min	1
Initial Activation	95°C	2 min	1
2-step cycling			
Denaturation	95°C	5 s	40
Annealing/ Extension*	60°C	30 s	

* Fluorescence data collection, approximate run time 67 min (Mx3005P®, Agilent Technologies, Inc.)

Data analysis and interpretation

Interpretation of results

For the assay to be valid the Positive Control must give a signal in the FAM and HEX channels with a C_T[1] < 35. The Negative Control must give no signal.

The following results are possible if working with unknown samples. The possible sample results are also summarized in Table 4 on page 17.

The sample is positive for Influenza A Virus, and the assay is valid, if the following criteria are met:

- The sample yields a signal in both, the FAM and the HEX channel.
- The Positive Control yields a signal in both, the FAM and the HEX channel.
- The Negative Control does not yield a signal in the FAM and HEX channels.

Note that very high concentrations of Influenza A Virus RNA in the sample may lead to a reduced HEX signal or no HEX signal due to competition with the Internal Control.

[1] Threshold cycle (C_T) — cycle at which the amplification plot crosses the threshold, i.e., there is the first clearly detectable increase in fluorescence

The sample is negative for Influenza A Virus, and the assay is valid, if the following criteria are met:

- The sample yields a signal in the HEX channel but not in the FAM channel.
- The Positive Control yields a signal in both, the FAM and the HEX channel.
- The Negative Control does not yield a signal in the FAM and HEX channels.

A positive HEX signal means that extraction and amplification were successful as the housekeeping gene (β-actin) within the sample is amplified.

The sample results are inconclusive, and the assay is invalid, if the following criteria are met:

- The sample yields no signal in the FAM and HEX channels.

If no signal is detected in the FAM (Influenza A Virus) and the HEX (endogenous Internal Control, EC) channel, the result is inconclusive. The absence of a signal for the housekeeping gene indicates strong PCR inhibition and/or other malfunctions, e.g., during extraction.

To check for inhibition, we recommend 1:5 dilution of the sample RNA in nuclease-free water, to repeat the RNA extraction procedure, or repeat the whole test procedure starting with new sample material.

Check that there is a fluorescence signal in all the channels for the positive control reaction (Positive Control). Absence of a signal for the Positive Control indicates an error, which could be due to incorrect setup of the reaction mix or incorrect cycling conditions.

Table 4. Results interpretation table*

Sample result	FAM (Influenza A Virus)	HEX (EC)
Influenza A Virus positive	X	X
Influenza A Virus strong positive	X	
Influenza A Virus negative		X
Inconclusive		

* Interpretation of sample results can be determined provided positive and negative control reactions are performed. The Positive Control must yield a signal in the FAM and HEX channels. The Negative Control must yield no signal in any channel.
For a complete explanation of possible sample results please refer to “Data analysis and interpretation” on page 15.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.

Visit **www.indical.com** for more information about afosa, bactotype, cador, cattletype, flocktype, pigtype, Svanovir and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual.

Notes

Limited License Agreement for virotype Influenza A 2.0 RT-PCR Kit

Use of this product signifies the agreement of any purchaser or user of the product to the following terms:

1. The product may be used solely in accordance with the protocols provided with the product and this handbook and for use with components contained in the kit only. INDICAL grants no license under any of its intellectual property to use or incorporate the enclosed components of this kit with any components not included within this kit except as described in the protocols provided with the product, this handbook, and additional protocols available at www.indical.com. Some of these additional protocols have been provided by INDICAL users for INDICAL users. These protocols have not been thoroughly tested or optimized by INDICAL. INDICAL neither guarantees them nor warrants that they do not infringe the rights of third-parties.

2. Other than expressly stated licenses, INDICAL makes no warranty that this kit and/or its use(s) do not infringe the rights of third-parties.

3. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

4. INDICAL specifically disclaims any other licenses, expressed or implied other than those expressly stated.

5. The purchaser and user of the kit agree not to take or permit anyone else to take any steps that could lead to or facilitate any acts prohibited above. INDICAL may enforce the prohibitions of this Limited License Agreement in any Court, and shall recover all its investigative and Court costs, including attorney fees, in any action to enforce this Limited License Agreement or any of its intellectual property rights relating to the kit and/or its components.

For updated license terms, see www.indical.com.

Change index

Handbook	Version	Change
HB-2577-EN-001	September 2022	Product launch

INDICAL BIOSCIENCE

Ordering: **www.indical.com/contact**
Technical Support: **support@indical.com**
Website: **www.indical.com**

Certificate of Analysis

ThermoFisher SCIENTIFIC

VetMAX™-Gold AIV Detection Kit

Product No. **4485261**
Lot No. **2311061**
Expiration Date **19OCT2024**

The VetMAX™-Gold AIV Detection Kit is a USDA-licensed real-time reverse transcription PCR assay for the detection of Avian Influenza Virus (AIV) RNA isolated from poultry oropharyngeal/tracheal samples. The assay is a single-tube, real-time RT-PCR in which RNA is reverse transcribed into cDNA. AIV targets are amplified and detected in real time using fluorescent TaqMan® probes.

TEST		SPECIFICATION	RESULT	
Real-Time RT-PCR amplification of AIV RNA transcript and Xeno™ RNA internal positive control. (RNA transcript stocks quantified by UV spectrophotometry)	No Template Control	No amplification	Pass	
	Low Potency sample 1600 copies AIV transcript per reaction	27.5-31.5 Ct	31.2	Pass
	Medium Potency sample 80,000 copies AIV transcript per reaction	22.0-26.0 Ct	24.9	Pass
	High Potency sample 4,000,000 copies AIV transcript per reaction	16.0-20.0 Ct	19.4	Pass
	AIV RNA control (from AIV-XenoRNA control mix) 8000 copies AIV RNA control per reaction	25.0-29.0 Ct	26.1	Pass
	XenoRNA control (from AIV-XenoRNA control mix) 8000 copies XenoRNA control per reaction	25.0-29.0 Ct	25.7	Pass
	XenoRNA control component 80,000 copies XenoRNA control per reaction	21.5-25.5 Ct	23.3	Pass

For Veterinary Use Only

Manufactured in compliance with our ISO 9001 and ISO 13485 certified quality management system.

Site: Austin, Texas, USA

Miranda Cooper
Digitally signed by Miranda Cooper
Date: 2023.12.11 13:19:22 -06'00'

Quality Assurance
Issued 11DEC2023

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Custom Primers and TaqMan® Probes shipped at ambient temperature reduce environmental impact and retain their quality and stability

Abstract

To minimize the adverse environmental impact of packaging and shipping products on gel or dry ice, Thermo Fisher Scientific investigated the feasibility of shipping its Custom Primers and TaqMan® Probes at ambient temperatures. This report describes stability testing of dye-labeled primers and MGB, TAMRA™, and QSY® probes after subjecting them to simulated summer shipping conditions. Analytical and stability testing demonstrated that Custom Primers and TaqMan® Probes that underwent simulated summer ambient-temperature shipping conditions maintained the same integrity and functionality as primers and probes that were kept at the recommended storage condition. By shipping at ambient temperatures, the need for expanded polystyrene (EPS) coolers and added refrigerant is eliminated and the fuel consumption and greenhouse gas emissions from transporting the product are significantly reduced.

Introduction

The adverse environmental impact of shipping refrigerated or frozen products is tremendous. The annual carbon footprint to manufacture EPS and convert it into coolers for our Custom Primers and TaqMan® Probes is approximately 6 tons CO_2-equivalents (CO_2e) [1]. Factoring in the number of shipments, the average distance traveled per package, and the fact that most packages are shipped via air, the annual total carbon footprint for transporting Custom Primers and TaqMan® Probes is 32 tons CO_2e [2].

There are other facts to consider beyond the greenhouse gas emissions. When a cooler arrives at the laboratory, the researcher is often put in the untenable position of deciding whether to burn additional fossil fuels to transport the empty cooler across country for reuse/recycling or to dispose of the cooler in a landfill. The best way to address the total environmental impact of "cold-chain" transport is to follow the hierarchy of "reduce, reuse, recycle": 1) Design the product for stability to ensure it can withstand the rigors of ambient shipping conditions without added refrigerant or insulation; 2) Design the packaging to be reusable, without increasing source material consumption; and 3) Recycle locally. We have opted to reduce whenever possible, reuse when it is an environmentally preferable option, and to encourage our customers to recycle locally.

Thermo Fisher Scientific has been systematically evaluating novel ways to minimize the impact of shipping Life Technologies™ products on gel or dry ice, and the CO_2 footprint left by these products during distribution. Here we demonstrate that selected Custom Primers and TaqMan® Probes are stable at ambient temperatures during shipping. By avoiding the cooler and refrigerant, the product can be shipped in a smaller, corrugated cardboard box, which improves the carrier's freight density (less fuel and emissions per box) and reduces the amount of packaging materials requiring disposal or recycling. By eliminating the cooler and gel or dry ice for these products, Thermo Fisher Scientific is helping to divert an annual total of nearly 1,826 kg (5,062 cubic feet)

life technologies

of EPS from landfills and incinerators by replacing it with recyclable corrugated paper packaging, and to reduce the annual total carbon footprint by 38 tons CO_2e [1,2].

In 2009, we investigated the stability of five TaqMan® Assays: TaqMan® Gene Expression, Custom TaqMan® Gene Expression, TaqMan® MicroRNA, TaqMan® Drug Metabolism Genotyping, and TaqMan® SNP Genotyping Assays [3]. These assays comprise a preformulated set of unlabeled locus-specific oligonucleotide primers and minor grove binder–nonfluorescent quencher (MGB-NFQ) probes labeled with a fluorescent dye (VIC® or FAM™ dye), and are supplied in liquid form. A total of 42 different TaqMan® Assays were selected to represent the widest range of performance as well as chemical, sequence, and structural motifs. Assays were subjected to simulated summer ambient shipping conditions and subsequently analyzed for physical integrity and functional performance. Stressed samples were compared to controls in analytical HPLC and functional real-time PCR assays. In all cases, simulated ambient shipping of the assays had no effect on their quality, integrity, or functional performance. This study provided ample evidence for the stability of a wide range of structural motifs and oligonucleotide sequences under ambient shipping conditions and also demonstrated the stability of the VIC® and FAM™ dyes and the MGB moiety at the concentrations found in the assays.

For many years, Custom Primers and TaqMan® Probes have been shipped refrigerated on gel ice (with storage after shipping at +4°C or –20°C, depending on the product). Building on our 2009 study, this paper describes results from stability testing carried out after the Custom Primers and TaqMan® Probes were exposed to established summer shipping temperature profiles. These experiments demonstrate that by shipping selected Custom Primers and TaqMan® Probes under ambient conditions, not only can we supply researchers with the same superior-quality product they are used to receiving, but we can also reduce our environmental footprint in the process. This is a win for our customers (eliminating packaging waste and extra costs associated with refrigerated shipments), a win for our planet (reducing resource consumption and total carbon footprint), and a win for our company (eliminating the need to manage cold-chain transport).

Materials and methods

Products tested. Custom Primers are 5'-labeled oligos that come with a choice of six dyes: 6-FAM™, TET™, VIC®, HEX™, NED™, or PET™ dye. The Custom Primer Pairs also come with an unlabeled oligo in a separate tube. Primers and Primer Pairs may be HPLC purified and can be ordered in two or three different quantities, with the largest having the highest concentration. For this study, four different labeled primers at the highest concentration were selected to represent the variety of primer types and dyes available (Table 1). The FAM™ and VIC® dyes were not tested with the primers because the 2009 study demonstrated the stability of these dyes in the TaqMan® Assays under ambient shipping conditions. Additionally, the Sequence Detection Primers, which are unlabeled, were not tested because the 2009 study established that unlabeled oligos are not affected by simulated ambient shipping conditions. All primers tested were formulated in Tris-EDTA (TE) buffer and were not HPLC purified. HPLC purification has no impact on the stability of the oligo or dye. Formulations in water were not evaluated because the pH of Tris buffers is known to vary inversely with temperature [4,5], something that does not occur in water, making TE a higher-risk formulation for ambient shipping.

TaqMan® MGB Probes incorporate a 5' reporter dye (FAM™, VIC®, TET™, or NED™ dye) and a 3' nonfluorescent quencher, with the MGB moiety attached to the quencher molecule. The TAMRA™ probes incorporate a 5' reporter dye (FAM™, TET™, or VIC® dye) and a 3' TAMRA™ quencher dye. The TaqMan® QSY® Probes can be ordered with a 5' reporter dye (FAM™, VIC®, ABY®, or JUN® dye) and the QSY® quencher. All TaqMan® Probes are HPLC purified and supplied at a single concentration in TE buffer. The MGB, TAMRA™, and QSY® probes were each tested with their respective dyes, with the exception of the MGB probe. This probe was not tested with VIC® dye because our own unpublished studies have shown that FAM™ is more labile than VIC® at elevated temperatures; therefore, FAM™ was used to represent a "worst-case" scenario. Because the 2009 study showed that variation in sequence and length did not affect oligo stability, a single sequence was chosen for all primers and probes:

5' - TGGACAGCCACCGACGAGAGCCTGG - 3'

Table 1. Custom Primers and TaqMan® Probes represented in this study.

Product Description	Reporter dye	Cat. No.
Custom Primers		
Sequence Detection Primers, 10,000 pmol, 80,000 pmol, 130,000 pmol	None	4304970, 4304971, 4304972
Custom 5'-Labeled Primer Pair Di-Repeats, 10,000 pmol, 80,000 pmol, **300,000 pmol**	HEX™, **NED™**, PET®, 6-FAM™, VIC®, TET™	4304976, 4304977, **4304978**
Custom 5'-Labeled Primer, 10,000 pmol, 80,000 pmol, **300,000 pmol**	HEX™, NED™, **PET®,** 6-FAM™, VIC®, TET™	450007, 450006, **450017**
Custom 5'-Labeled Primer Pair, 10,000 pmol, 80,000 pmol, **300,000 pmol**	HEX™, NED™, PET®, 6-FAM™, VIC®, **TET™**	450056, 450059, **450062**
Custom 5'-Labeled Primer Pair Di-Repeat + Tail, 10,000 pmol, 80,000 pmol, **300,000 pmol**	**HEX™**, NED™, PET®, 6-FAM™, VIC®, TET™	4304979, 4304981, **4304982**
TaqMan® Custom Probes		
TaqMan® MGB Probe, 6,000 pmol, 20,000 pmol, **50,000 pmol**	**6-FAM™**, VIC®, **NED™, TET™**	4316034, 4316033, **4316032**
TaqMan® TAMRA™ Probe, 6,000 pmol, 20,000 pmol, **50,000 pmol**	**VIC®, 6-FAM™, TET™**	450025, 450024, **450003**
TaqMan® QSY® Probe, 6,000 pmol, 20,000 pmol, **50,000 pmol**	**6-FAM™, VIC®, ABY®, JUN®**	4482777, 4482778, **4482779**

Products tested are in **bold**

Creating replicates. To help eliminate manufacturing lot variability when creating the replicates, individual tubes of the primers and probes were manufactured, pooled, and aliquoted into the same packaging tube at the same fill volume as specified for the manufactured product. A total of 10 lots for each primer and probe were used to create five replicate stress tubes and five replicate control tubes. The control tubes were kept at –20°C for the duration of the study.

Simulated shipping conditions. To simulate temperatures experienced during shipping, samples were placed in a cycling environmental chamber (Thermotron® S-16) programmed to reproduce a "worst-case" 288-hour (12-day) summer temperature profile (Figure 1). This profile is adopted from one developed and validated by Amgen to simulate global ambient shipping conditions and mimics product temperature extremes encountered during transit of over 2,500 shipments during summer months between the latitudes of 59.9° N and 37.8° S [6]. Testing of winter ambient conditions was not considered, due to the low risk of exposing the Custom Primers and TaqMan® Probes to cold conditions.

Stability and integrity testing. Structural integrity changes in stressed samples compared to controls were measured by reverse-phase HPLC (RP-HPLC) and MALDI mass spectrometry. RP-HPLC samples were analyzed using an Agilent® 1200 HPLC. The HPLC column used was a Phenomenex® Clarity® 3 µm Oligo-RP, 2.0 mm ID x 50 mm. Mobile phases used were 0.1 M TEAA (triethylamine acetate) in water and 0.1 M TEAA in

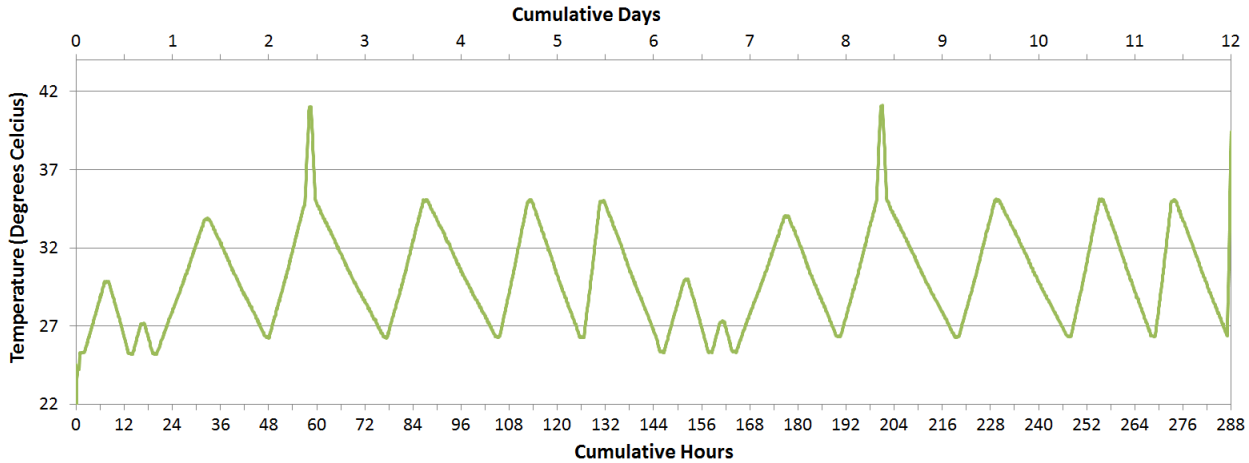

Figure 1. 288 hr summer temperature profile used to simulate shipping conditions. The summer temperature profile was used to mimic average high temperature extremes between the latitudes of 59.9° N and 37.8° S.

50% water/40% acetonitrile/10% methanol for the primers, MGB, and TAMRA™ probes, and 0.1 M TEAA in water and 2.0 M TEAA in 5% water/95% methanol for the QSY® probes. Absorbance was monitored at 260 nm for the oligonucleotide and at the maximum absorbance wavelength of the dye. Samples for MALDI mass spectrometry were analyzed on an AB Sciex® 4800 Plus MALDI TOF/TOF™ Analyzer.

Results

RP-HPLC. RP-HPLC was used to create peak profiles of the dye-labeled primers using UV/Vis absorbance detection. Matched test and control tubes from each assay were analyzed. An example of the data is shown in Figure 2. Test and control peak profiles were compared, and the purity (peak areas) were calculated (data not shown). For all samples analyzed, test samples were judged as identical to matched controls (no degradation), confirming that the simulated shipping stress did not affect product integrity.

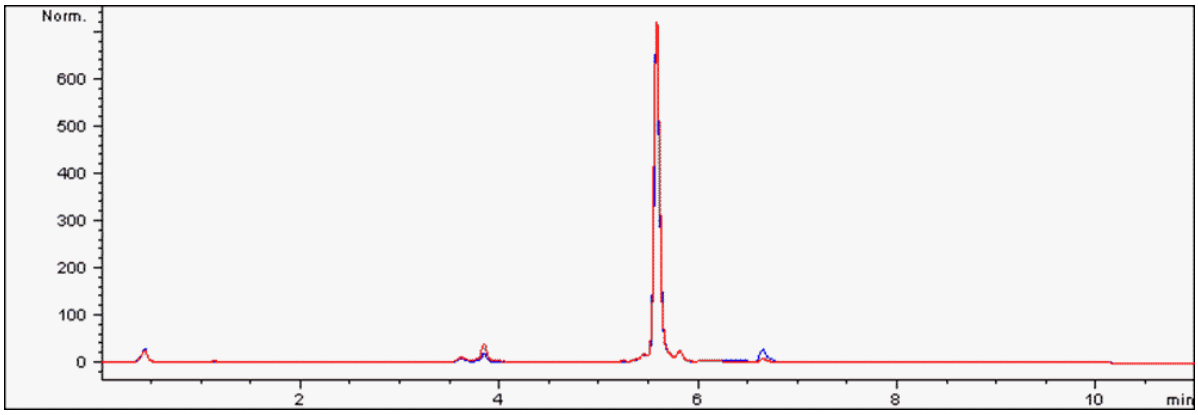

Figure 2. Simulated summer ambient shipping does not affect oligonucleotide stability—representative HPLC data. The effect of simulated summer ambient shipping on oligonucleotide integrity was measured by comparing RP-HPLC profiles of matched test and control samples. The HPLC chromatogram profiles of the test samples are comparable to the profiles of the control samples. There was no indication of probe or primer degradation in the simulated ambient-shipped 5'-Labeled Primer Pair Di-Repeats with the NED™ dye (red) compared to the matched control (blue).

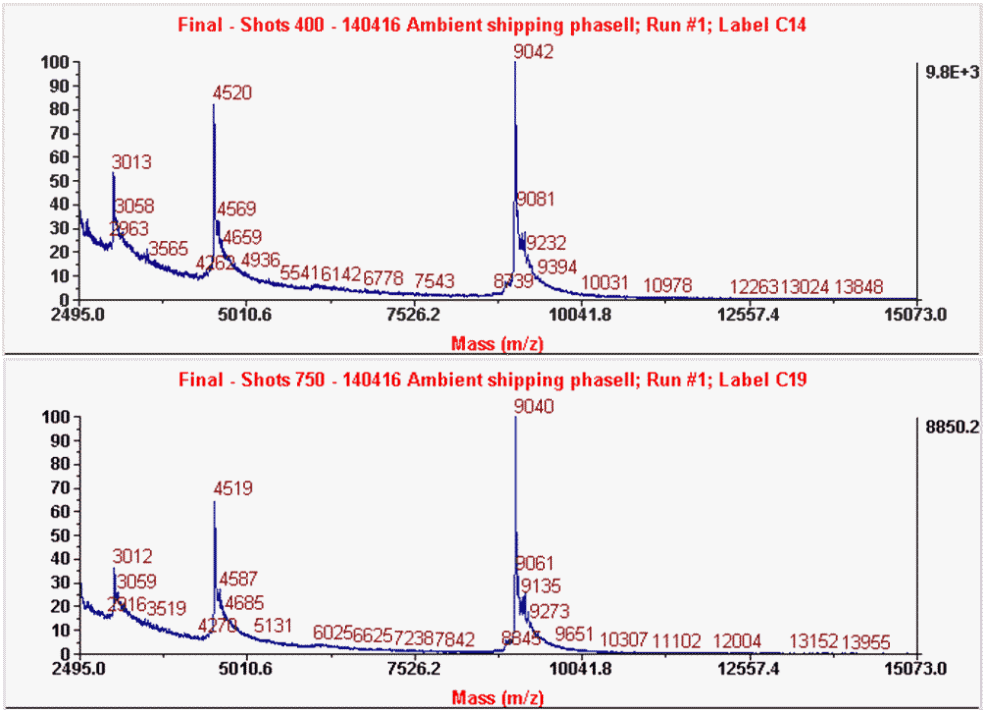

Figure 3. Simulated summer ambient shipping does not affect oligonucleotide stability—representative MALDI mass spectrometry data. The effect of simulated summer ambient shipping on oligonucleotide integrity was measured by comparing mass spectrum profiles of matched test and control samples. The profiles of the test samples are comparable to the profiles of the control samples. There was no indication of probe or primer degradation in the simulated ambient-shipped TaqMan® TAMRA™ Probe with the VIC™ dye (bottom) compared to the matched control (top).

MALDI mass spectrometry. MALDI mass spectrometry was used to generate mass profiles of the dye-labeled primers and probes. Again, matched test and control assays were analyzed and compared to each other. An example mass spectrum is shown in Figure 3. Test and control samples showed the same mass profiles, indicating that no degradation of the oligo, dye, or quencher occurred during the shipping simulation, further confirming that the simulated shipping stress did not affect product integrity.

Conclusions

The data described in this paper demonstrate that ambient shipping conditions have no effect on the quality and stability of Custom Primers and TaqMan® Probes. For each dye-labeled primer and probe tested, we were able to clearly demonstrate that ambient-temperature shipping conditions do not affect the product quality or integrity.

These results substantiate the change to ambient shipping conditions, and provide the researcher with confidence that when shipped under ambient conditions, their Custom Primers and TaqMan® Probes will exhibit no difference in function or stability compared to dry or gel ice–shipped products. In addition to ensuring our customers will continue to receive the highest quality possible, this study enables us to reduce the impact of transport of these products by 32 tons CO_2e. Our customers will see a reduction of 1,826 kg of EPS waste. Our planet will collectively see CO_2 emissions reduced by 38 tons every year.

References

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Find out more at **lifetechnologies.com/ecotaqman**

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A Thermo Fisher Scientific Brand

AgPath-ID™ One-Step RT-PCR Reagents

USER GUIDE

Core reagents for one-step qRT-PCR detection of pathogens

Catalog Numbers AM1005, 4387424, and 4387391

Publication Number 1005M

Revision J

For Veterinary Use Only.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

Revision history: Pub. No. 1005M

Revision	Date	Description
J	27 August 2019	• Updated to the current document template, with associated updates to the warranty, trademarks, and logos. • Removed Detection Enhancer from "Contents and storage" on page 4.
H	March 2015	• Clarified that the kit is intended for use with single or duplex assays. • Other format, style, and legal updates.
G	October 2012	Baseline for this revision history.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

AgPath-ID™ One-Step RT-PCR Reagents are designed for sensitive, robust amplification of RNA targets using a single-tube TaqMan®-based real-time RT-PCR strategy. Each kit is optimized for use with single or duplex TaqMan® primer/probe sets.

Each kit includes the following reagents:

- **25X RT-PCR Enzyme Mix:** Contains ArrayScript™ Reverse Transcriptase and AmpliTaq Gold™ DNA Polymerase.
- **2X RT-PCR Buffer:** Includes ROX™ passive reference dye for quantitative fluorescent signal normalizaton.

For higher order multiplexed assays or samples that have been extracted from matrices with high inhibitor content (such as fecal samples and oral fluids), use the Path-ID™ Multiplex One-Step RT-PCR Kit (Cat. No. 4442136).

Contents and storage

Component	Cat. No. AM1005 (100 reactions)	Cat. No. 4387424 (500 reactions)	Cat. No. 4387391 (1,000 reactions)	Storage
2X RT-PCR Buffer	1375 µL	7 mL	14 mL	–10°C to –30°C in a non-frost-free freezer
25X RT-PCR Enzyme Mix	110 µL	550 µL	1100 µL	
Nuclease-free water	1.75 µL	25 mL	25 mL	—

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Real-time PCR system, one of the following:	
QuantStudio™ 5 Real-Time PCR System	Contact your local sales office.
Applied Biosystems™ 7500 Fast Real-Time PCR System	
Applied Biosystems™ 7500 Real-Time PCR System	
Applied Biosystems™ 7900HT Fast Real-Time PCR System	
ABI PRISM™ 7500 Sequence Detection System	
MX3000P QPCR System	Agilent
SmartCycler™ II System	Cepheid
Equipment	
Adjustable pipettors	MLS
Reagents	
***(Optional)* Detection Enhancer**[1]	
Detection Enhancer - 100 rxn	A44810
Detection Enhancer - 500 rxn	A44941
Detection Enhancer - 1000 rxn	A44811
Tubes, plates, and other consumables	
96-well PCR plate or tubes	thermofisher.com/plastics
Nuclease-free pipette tips	thermofisher.com/pipettetips
Reagent reservoirs or tubes[2]	MLS

[1] Recommended for targets with high GC content and/or persistent secondary structure.
[2] Recommended for preparing the RT-PCR master mix.

Guidelines for input RNA

- Use pure RNA that is free of RT-PCR inhibitors.
- We recommend using a MagMAX™ RNA isolation kit that is appropriate for your sample type. Go to **thermofisher.com**, then search for ***MagMAX***.
- For cell-free sample types, such as serum, we recommend using the MagMAX™ CORE Nucleic Acid Purification Kit (Cat. No. A32700 or A32702).

Guidelines for PCR primer/TaqMan® probe mix

- Single and duplex TaqMan® primer/probe sets must be compatible with your real-time PCR instrument and designed for one-step RT-PCR.
- Optimize the concentrations of primers and probe for your experiment. See Table 1 for recommended starting concentrations.
- The reverse transcriptase that is contained in the kit is produced using an *E. coli* expression vector. The vector contains a proprietary version of the MMLV *pol* gene (GenBank™ accession no. J02255) expressed from pET-24(+). It is possible that a minimal amount of the expression vector could be carried over into the final master mix formulation. If you are targeting MMLV, a related virus, or any of the plasmid sequences, we recommend designing primer sequences that are not contained in the expression vector.

Table 1 Recommended PCR Primer/TaqMan® probe concentrations

Component	Final concentration in the reaction	25X primer/probe mix[1]
Forward PCR primer	400 nM	10 µM
Reverse PCR primer	400 nM	10 µM
TaqMan® probe	120 nM	3 µM

[1] Use 1 µL of PCR primer/TaqMan® probe mix per 25-µL RT-PCR reaction.

2 Methods

Set up the real-time PCR instrument

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions.

Set up the thermal protocol for your instrument according to one of the following tables.

- Passive reference dye: ROX™ (included in the RT-PCR Buffer)
- Reaction volume: 25 µL

IMPORTANT! If you are using the SmartCycler™ II instrument, set ramp rates to 1.6°C/sec to prevent amplification failure.

Table 2 Thermal protocol: SmartCycler™ II instrument

Step	Stage	Cycles	Temp	Time
Reverse transcription	1	1	45°C[1]	10 min
RT inactivation/initial denaturation	2	1	95°C	15 min
Amplification	3	40	95°C 60°C	15 sec 60 sec

[1] 50°C may be a more effective RT temperature for some PCR primer sets.

Table 3 Thermal protocol: All other compatible instruments

Step	Stage	Cycles	Temp	Time
Reverse transcription	1	1	45°C	10 min
RT inactivation/initial denaturation	2	1	95°C	10 min
Amplification	3	40	95°C 60°C	15 sec 45 sec

Prepare the RT-PCR reactions

- Calculate the number of required reactions. Scale reaction components based on the single-reaction volumes, then include 5–10% overage, unless otherwise indicated.
- Include duplicate no template controls (NTCs) using nuclease-free water in place of sample.

1. Prepare each RT-PCR master mix on ice according to Table 4.
2. Transfer the appropriate volume of RT-PCR master mix to a PCR plate or tubes.
3. Add samples or controls to the wells or tubes containing RT-PCR master mix (25 µL final volume per reaction).

Table 4 RT-PCR reaction mix volumes

Component		Volume
RT-PCR master mix	2X RT-PCR Buffer	12.5 µL
	Forward and reverse PCR primers	__ µL
	TaqMan® probes	__ µL
	25X RT-PCR Enzyme Mix	1 µL
	(Optional) Detection Enhancer[1]	(1.67 µL)
RNA sample (or nuclease-free water for NTCs)		__ µL
Total volume per reaction		**25 µL**

[1] Run the first reaction without Detection Enhancer. Detection Enhancer is recommended only for targets with high GC content and/or persistent secondary structure. Detection Enhancer can compromise sensitivity for other targets. For more information, see Appendix A, "Troubleshooting".

Perform RT-PCR, then analyze the results

Start the run, then analyze the RT-PCR data according to the PCR instrument manufacturer's instructions.

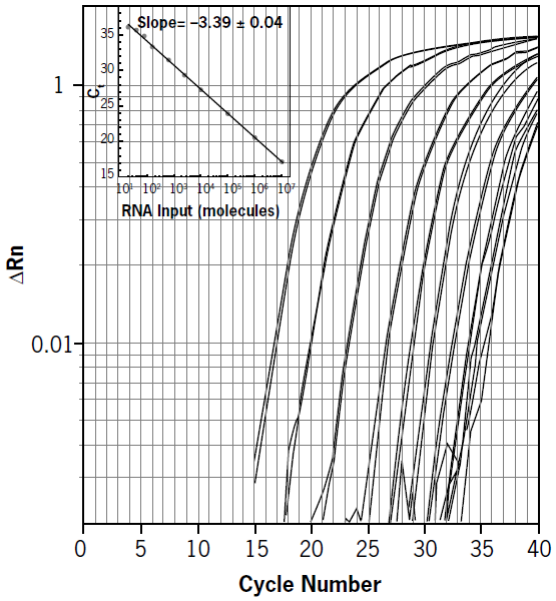

Figure 1 Amplification of a control RNA sequence using AgPath-ID™ One-Step RT-PCR Reagents

For the amplification, 5 µL of Xeno™ RNA-01 Control dilutions, containing 10^7, 10^6, 10^5, 10^4, 2500, 640, 160, 80, 40, and 20 RNA molecules, were amplified using the kit on an Applied Biosystems™ 7900HT Fast Real-Time PCR System. The amplification plots are shown with an inset that displays the linear relationship between C_t and RNA input; the slope is ~3.39, which indicates ~100% amplification efficiency.

A

Troubleshooting

Observation	Possible cause	Recommended action
No signal from samples that are expected to be positive	The target sequence has high GC content or persistent secondary structure.	Include Detection Enhancer in the RT-PCR master mix to improve amplification. Add 1.67 µL of Detection Enhancer per 25-µL reaction.
	Inhibitors are present in the RNA sample.	Isolate RNA using the appropriate MagMAX™ kit. MagMAX™ kits are typically more effective than glass fiber filter-based RNA isolation methods or TRI Reagent™.
		For samples that contain minimal amounts of inhibitors, decrease the volume of sample added to the reaction, then repeat RT-PCR.
		Dilute the sample 5- or 10-fold, then repeat RT-PCR.
	There were problems with the RNA isolation procedure.	For viral RNA isolation, if a carrier RNA was used, check the concentration of the carrier RNA to evaluate its recovery.
	For user-designed assays, the assay concentration was improperly optimized with RT-PCR Buffer.	Optimize the assay concentration.
	The thermal cycler was not programmed correctly.	Check the thermal protocol on the instrument to ensure that the appropriate settings are configured.
	The RT-PCR master mix was not prepared correctly.	Prepare new RT-PCR master mix, then repeat RT-PCR.
	25X RT-PCR Enzyme Mix was stored improperly and lost activity.	Store reagents as directed.
Signal detected in no template control (NTC)	Contamination occurred during RT-PCR.	Repeat the qRT-PCR reaction with fresh reagents and decontaminated pipettes.
		Follow "Good laboratory practices for PCR and RT-PCR" on page 11.
		It is possible that a minimal amount of the expression vector could be carried over into the final master mix formulation. If you are targeting MMLV, a related virus, or any of the plasmid sequence, we recommend designing primer sequences that are not contained in the expression vector.

B

Supplemental information

Good laboratory practices for PCR and RT-PCR

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).

- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.
- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Documentation and support

Customer and technical support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Master mixes to help meet your specific needs, sample type, and testing method

Enjoy the confidence that comes with using Applied Biosystems™ master mixes that veterinary research labs around the world have come to trust for reliability and robust performance. Whether you're working with RNA or DNA, performing singleplex or multiplex reactions, or testing simple or difficult samples, we have the optimal master mix that can work for you and help deliver robust and consistent results you can trust.

Applied Biosystems™ kits and master mixes

Fast-cycling multiplex one-step RT-PCR master mix	• VetMAX™ Fast Multiplex Master Mix
One-step RT-PCR master mix	• AgPath-ID™ One-Step RT-PCR Kit
Standard-cycling multiplex one-step RT-PCR master mix	• Path-ID™ Multiplex One-Step RT-PCR Kit
qPCR master mix	• Path-ID™ qPCR Master Mix (just for DNA)
Master mixes with internal positive control	• VetMAX™-Plus One-Step RT-PCR Kit • VetMAX™-Plus Multiplex One-Step RT-PCR Kit • VetMAX™-Plus qPCR Master Mix

applied biosystems

Fast multiplex master mix (with ROX)

VetMAX Fast Multiplex Master Mix—highly analytically sensitive master mix compatible with fast cycling conditions and optimized for inhibitory animal samples.

- Compatible with fast cycling (<50 minute thermal cycler run time)
- Compatible with a variety of assays and complexities (RNA, DNA, singleplex, multiplex)
- Ability to tolerate inhibitors from a variety of sample types such as feces, environmental samples, blood, milk, and oral fluid
- Single-tube format for ease of use

Formulation

Fast multiplex master mix containing:

- A concentrated reverse transcriptase enzyme capable of producing high cDNA yields
- Concentrated ultrapure hot-start DNA polymerase providing exceptional analytical specificity and sensitivity
- Fast cycling–optimized 2X RT-PCR buffer for inhibitor-tolerant, multiplex-compatible reverse transcription and PCR
- Includes ROX dye as an internal reference for normalization and precise data analysis

Performance

VetMAX Fast Multiplex Master Mix (with ROX) is a fast-cycling, multiplex-compatible master mix that has enhanced multiplexing capability, analytical sensitivity, and inhibitor tolerance. Figure 1 shows a comparison between a standard-cycling master mix and the VetMAX Fast Multiplex Master Mix for bovine viral diarrhea virus (BVDV) RNA. This master mix helps decrease time-to-results while providing higher-order multiplexing capabilities and enhanced inhibitor tolerance, so you can get information for up to four targets from a single reaction regardless of sample type. The single-tube format saves hands-on time—just add your assay and sample and you're ready to go.

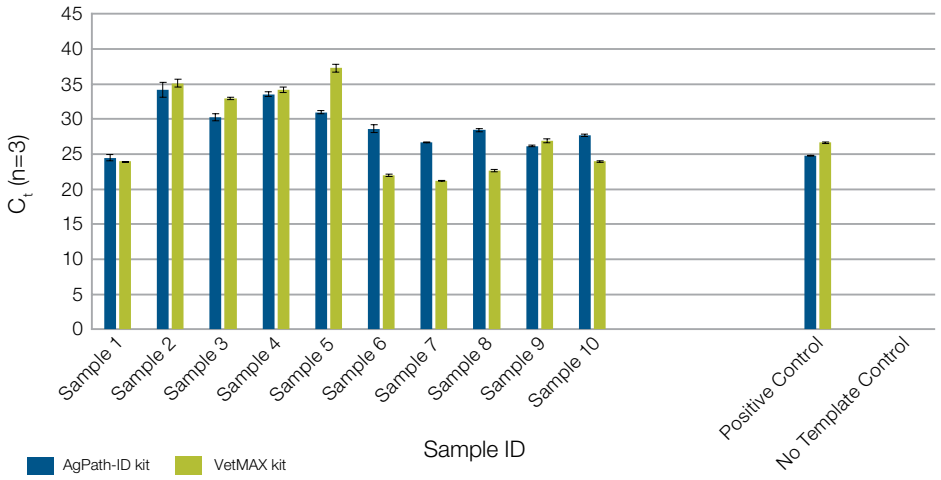

Figure 1. AgPath-ID One-Step RT-PCR Kit run on standard mode and VetMAX Fast Multiplex Master Mix (BVDV RNA) run on fast mode. These data show that the VetMAX kit has similar sensitivity compared with the AgPath-ID kit, with a significantly shorter runtime.

One-step RT-PCR master mix

AgPath-ID One-Step RT-PCR Kit—economical, high-quality, ready-to-use master mix for amplification of RNA targets.

- Consistent, reliable amplification helps provide results you can trust
- Simple single-tube, one-step reaction minimizes handling and helps reduce the risk of cross-contamination

Formulation

The AgPath-ID One-Step RT-PCR Kit is designed for analytically sensitive, robust amplification of RNA targets in the presence of PCR inhibitors typically found in animal samples. The kit includes:

- 25X RT-PCR enzyme mix containing:
  - Invitrogen™ ArrayScript™ Reverse Transcriptase (RT), a mutant reverse transcriptase enzyme RT that produces high cDNA yields
  - Ultrapure hot-start DNA polymerase providing exceptional analytical specificity and sensitivity
- Optimized 2X RT-PCR buffer for efficient, robust reverse transcription and PCR
  - Includes ROX dye as an internal reference for normalization and precise data analysis

Sensitive, reliable performance

To illustrate the consistent performance of the AgPath-ID One-Step RT-PCR Kit, serial dilutions of virus A control RNA containing 5 to 5 x 10^6 copies were amplified (Figure 2). The amplification plot shows a consistent set of curves expected from highly efficient PCR, and the graph shows the reliability and efficiency of the reaction across a wide range of input template amounts.

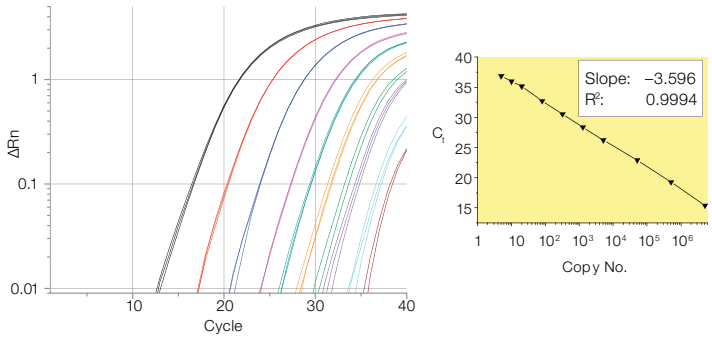

Figure 2. qRT-PCR targeting serially diluted virus A control RNA transcript (5 to 5 x 10^6 copies) demonstrates highly efficient and consistent performance of the AgPath-ID One-Step RT-PCR Kit.

Figure 3 shows amplification of a serial dilution of a different control RNA, virus B. Amounts of RNA were kept low (20 to 40,000 copies) in order to compare the analytical sensitivity of target amplification of the AgPath-ID kit and another supplier's RT-PCR kit. The AgPath-ID One-Step RT-PCR Kit provided earlier C_t values and better analytical sensitivity than the other supplier's kit across the dilution range.

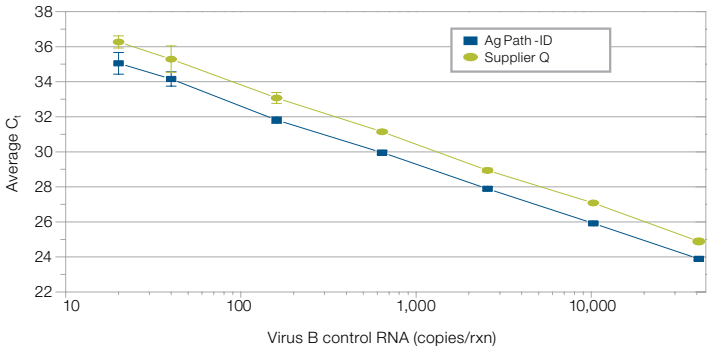

Figure 3. The AgPath-ID One-Step RT-PCR Kit is more sensitive than another leading supplier's kit. Serially diluted virus B control RNA (20 to 40,000 copies) was amplified using the AgPath-ID One-Step RT-PCR Kit and another supplier's kit.

Multiplex one-step RT-PCR master mix

Path-ID Multiplex One-Step RT-PCR Kit—highly analytically sensitive and convenient master mix optimized for veterinary labs targeting RNA pathogens.

- Simultaneous multiplex amplification of up to four different targets helps save time and money
- Optimized to amplify low–copy number (20 copies) targets to deliver results even with challenging samples
- Capable of amplification of over seven logarithmic units of input to provide robust performance when you need it

Formulation

The Path-ID Multiplex One-Step RT-PCR Kit is designed for the analytically sensitive, robust amplification and multiplex quantitation of animal pathogen RNA in a simple format. The kit includes:

- Multiplex enzyme mix containing:
  - A concentrated reverse transcriptase enzyme capable of producing high cDNA yields
  - Ultrapure hot-start DNA polymerase providing exceptional analytical specificity and sensitivity
- Multiplex RT-PCR buffer with optimized reagents for efficient, robust results from both the reverse transcription reaction and the PCR
  - Includes ROX dye as an internal reference for normalization and precise data analysis

Multiplex with confidence

In the study depicted in Figure 4, the Path-ID Multiplex One-Step RT-PCR Kit provides higher target analytical sensitivity in comparison to another supplier's product.

Figure 5 shows that the Path-ID Multiplex One-Step RT-PCR Kit comparably amplifies targets in singleplex and duplex RT-PCR reactions, suggesting that there is no loss of sensitivity as a result of multiplexing.

Figure 6 shows the amplification of four targets by multiplex RT-PCR using the Path-ID Multiplex One-Step RT-PCR Kit. The quantities of three of the targets in the experiment were held constant, but the fourth target was serially diluted to show the dynamic range of multiplex target amplification with the kit. The results show that the Path-ID Multiplex One-Step RT-PCR Kit consistently amplifies four animal sample-derived RNA targets in a single reaction.

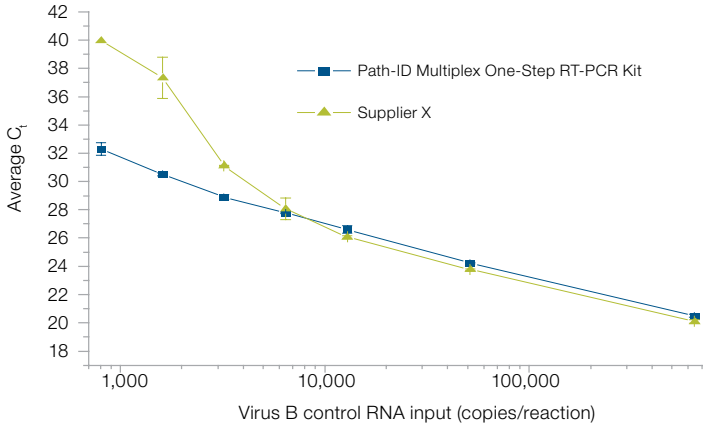

Figure 4. The Path-ID Multiplex One-Step RT-PCR Kit amplifies lower amounts of target with better analytical sensitivity (lower C_t values) than another supplier's kit. A quadruplex RT-PCR experiment was performed using the Path-ID Multiplex One-Step RT-PCR Kit and another supplier's kit. Only data for the virus B target are shown.

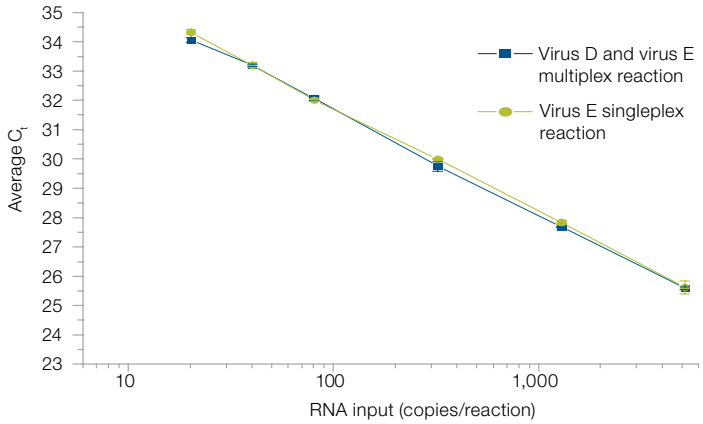

Figure 5. The Path-ID Multiplex One-Step RT-PCR Kit shows no difference in analytical sensitivity between singleplex and multiplex reactions. Virus E RNA was reverse-transcribed and PCR-amplified in a singleplex reaction, and virus D RNA and virus E RNA were reverse-transcribed and coamplified in a duplex reaction, using the Path-ID Multiplex One-Step RT-PCR Kit for both reactions.

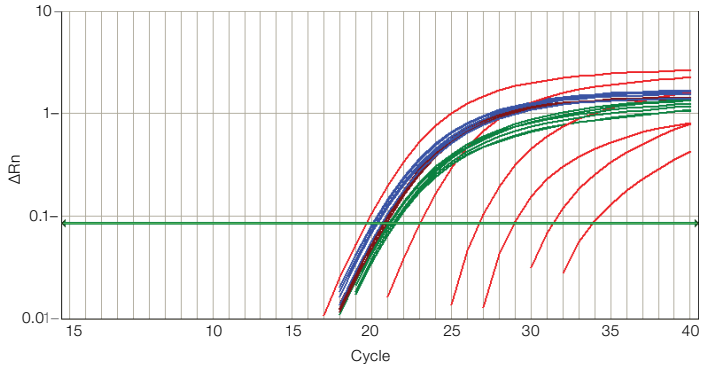

Figure 6. The Path-ID Multiplex One-Step RT-PCR Kit consistently amplifies multiple pathogen targets in a single reaction. Applied Biosystems™ Xeno™ RNA Control and control RNAs for virus A, virus B, and virus C were amplified in a single multiplex reaction using the Path-ID Multiplex One-Step RT-PCR Kit. A sample set with fixed amounts of three of the targets and a serial dilution series of the virus B control RNA (red curve) were included.

qPCR master mix

Path-ID qPCR Master Mix—highly sensitive master mix used to detect animal sample–derived DNA, optimized to perform in the presence of challenging qPCR inhibitors.

- Capable of amplifying over 7 logarithmic units of input and down to 25 copies of target for dependable, robust performance
- Inhibitor tolerance to help deliver accurate results even with challenging samples
- Stable performance at a wide temperature range allows for convenient reaction setup and reagent storage

Formulation

Path-ID qPCR Master Mix is designed for the sensitive, robust amplification of animal pathogen DNA in a convenient format. It includes:

- Ultrapure hot-start DNA polymerase to enable room-temperature reaction setup and minimize nonspecific PCR products
- Optimized buffer and dNTPs for enhanced analytical sensitivity and functionality in the presence of PCR inhibitors
- ROX dye as an internal reference for normalization and precise data analysis

Convenience and performance

The Path-ID qPCR Master Mix provides dependable target amplification over a linear dynamic range of 6 orders of magnitude, down to 25 copies of target (Figure 7). Path-ID qPCR Master Mix enables amplification of even the most dilute samples.

Path-ID qPCR Master Mix provides reliable amplification of numerous animal sample–derived DNA targets in the presence of PCR inhibitors frequently associated with agricultural samples. Figure 8 shows the ability of Path-ID qPCR Master Mix to tolerate high levels of both hematin (20 µM) and humic acid (15 ng/µL) compared to another supplier's master mix.

Path-ID qPCR Master Mix retains high performance even after exposure to harsh conditions. In Figure 9, Path-ID qPCR Master Mix was subjected to multiple freeze/thaw cycles as well as room-temperature treatment. In all cases, Path-ID qPCR Master Mix demonstrates equivalent amplification, exhibiting its stability during harsh storage events and even room-temperature reaction setup.

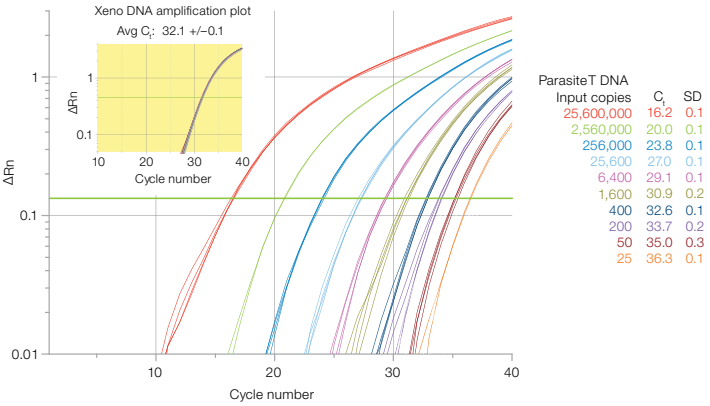

Figure 7. An amplification plot for parasite T DNA in four replicate reactions using Path-ID qPCR Master Mix demonstrates that even the most dilute samples are easily amplified. All reactions showed consistent amplification of Xeno DNA Control, an internal positive control (inset).

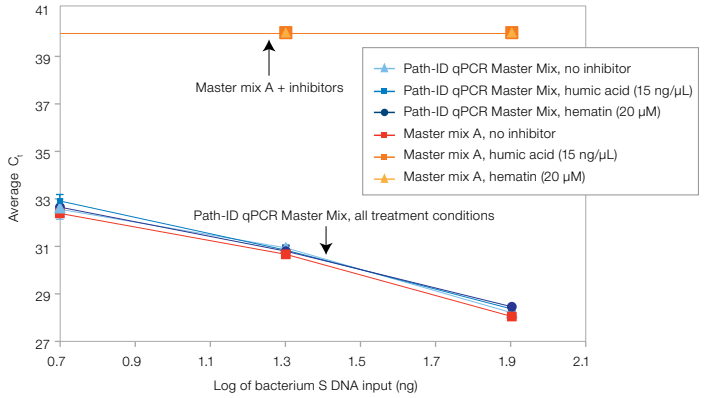

Figure 8. Path-ID qPCR Master Mix shows better tolerance to inhibitors than the competitor's master mix. C_t values are shown for amplification of a dilution series of bacterium S target DNA in the presence of PCR inhibitors, hematin (20 µM) and humic acid (15 ng/µL). The limit of detection for C_t is set at 40.

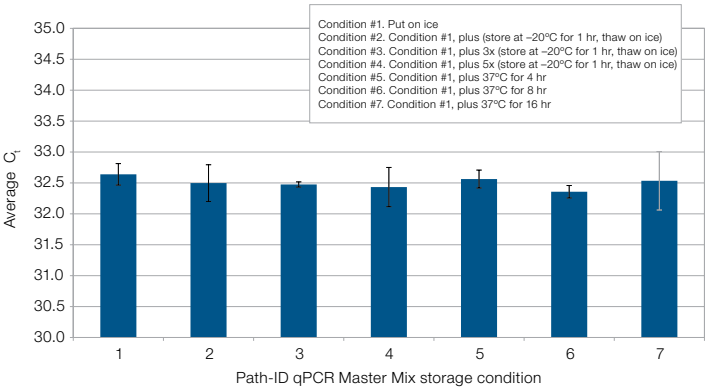

Figure 9. C_t values are given for amplification of bacterium M DNA using Path-ID qPCR Master Mix with various handling conditions. PCR was performed on bacterium M DNA using Path-ID qPCR Master Mix that had been subjected to various freeze/thaw cycles and stored at 37°C for different lengths of time.

Master mixes with internal positive control

Applied Biosystems™ VetMAX™-Plus master mixes provide the highly analytically sensitive and robust performance you need with the added confidence and convenience of a Xeno internal positive control (IPC). The use of an IPC in pathogen detection workflows allows you to distinguish true target negatives from PCR inhibition.

- Xeno IPC monitors the reaction for inhibition and effectiveness of nucleic acid purification, enabling greater confidence in results
- Formulations are optimized for use in detecting challenging sample–derived RNA or DNA
- A suite of master mix options (RT-PCR, multiplex, qPCR) is available to fit your unique application

Formulations

Components of each VetMAX-Plus kit are provided below.

VetMAX-Plus One-Step RT-PCR Kit

- 25X RT-PCR enzyme mix containing:
  - ArrayScript Reverse Transcriptase, a mutant reverse transcriptase enzyme that produces high cDNA yields
  - Ultrapure hot-start DNA polymerase providing exceptional analytical specificity and sensitivity
- 2X RT-PCR buffer for efficient, robust reverse transcription and PCR
  - Includes ROX dye as an internal reference for normalization and precise data analysis
- Xeno RNA Control

VetMAX-Plus Multiplex One-Step RT-PCR Kit

- 10X multiplex enzyme mix containing:
  - A concentrated reverse transcriptase enzyme capable of producing high cDNA yields
  - Ultrapure hot-start DNA polymerase providing exceptional analytical specificity and sensitivity
- 2X multiplex RT-PCR buffer for efficient, robust reverse transcription and PCR
  - Includes ROX dye as an internal reference for normalization and precise data analysis
- Xeno RNA Control

VetMAX-Plus qPCR Master Mix

- 2X qPCR master mix containing:
  - Ultrapure hot-start DNA polymerase that enables room-temperature reaction setup and minimizes nonspecific PCR products
  - Optimized buffer and dNTPs for enhanced sensitivity and functionality in the presence of PCR inhibitors
  - ROX dye as an internal reference for normalization and precise data analysis
- Xeno DNA Control

Qualified results

Using Xeno IPC effectively monitors for PCR inhibition, which means that you can easily qualify your testing results. Figure 10 shows how Xeno IPC identifies the presence of a PCR inhibitor (hematin) at multiple concentrations. The data show that Xeno IPC follows the target's trend of increasing C_t values due to inhibition and therefore can be used as an indicator of inhibition in the reaction. Since the expected range of Xeno IPC C_t values in a normal reaction (without inhibition) is known, you can determine the effect that inhibition has on the reaction, thereby lowering the risk of false negative results.

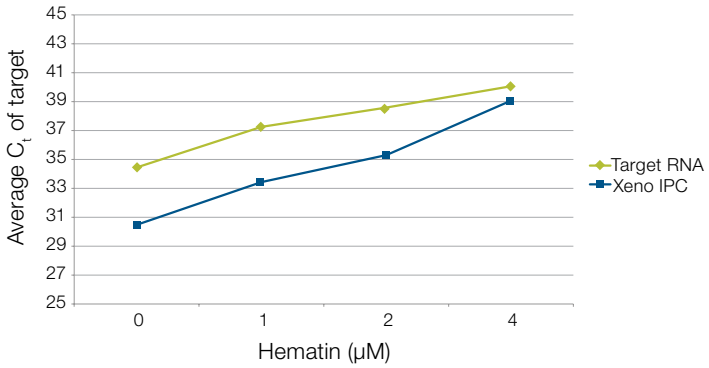

Figure 10. Graph depicting the effect of increasing inhibition on RNA target and subsequent effect on Xeno IPC. 100 copies per reaction of RNA target and 1,000 copies per reaction of Xeno IPC were exposed to increasing levels of hematin (0–4 µM).

For greater quality and consistency of sample-derived RNA and DNA detection, use VetMAX-Plus master mixes with Applied Biosystems™ VetMAX™ reagents and controls.

Ordering information

Product	Quantity	Cat. No.
Path-ID qPCR Master Mix	100 reactions	4388643
	500 reactions	4388644
AgPath-ID One-Step RT-PCR Kit	100 reactions	AM1005
	500 reactions	4387424
	1,000 reactions	4387391
Path-ID Multiplex One-Step RT-PCR Kit	100 reactions	4442135
	500 reactions	4442136
	1,000 reactions	4442137
VetMAX-Plus One-Step RT-PCR Kit	100 reactions	4415328
VetMAX-Plus Multiplex One-Step RT-PCR Kit	100 reactions	4415330
VetMAX-Plus qPCR Master Mix	100 reactions	4415327
VetMAX Fast Multiplex Master Mix	100 reactions	A57081
	500 reactions	A57305
	1,000 reactions	A57306

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COL27541 0423

Fast and Reliable: Single-Tube Real-Time RT-PCR

High-quality, ready-to-use reagents for amplification of animal pathogen RNA

- Obtain results in approximately 1 hour
- Consistently amplify RNA targets with high sensitivity
- Versatile design allows for use with multiple PCR cyclers, primers, and probes
- Minimize cross-contamination potential with fast and simple reaction setup

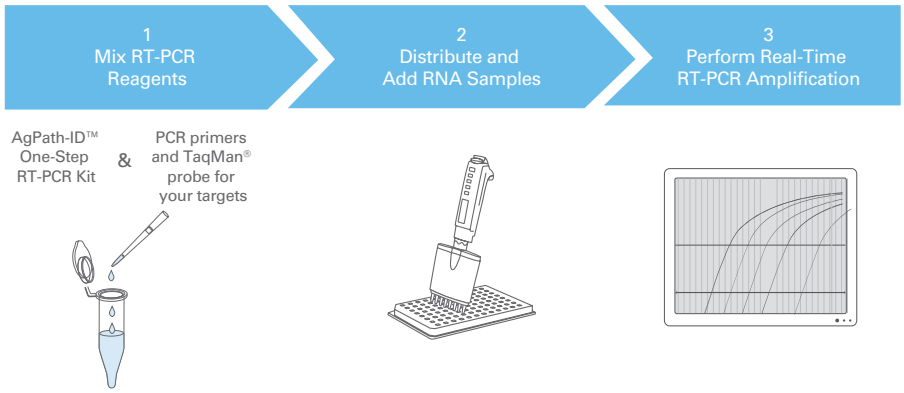

Figure 1. AgPath-ID™ One-Step RT-PCR Procedure Overview.

The AgPath-ID™ One-Step RT-PCR Kit is designed for sensitive, robust amplification of RNA targets using a rapid, single-tube real-time reverse transcription PCR (RT-PCR) strategy. You supply the PCR primers and TaqMan® probe (optional); the kit includes the buffers and enzymes needed for RT-PCR.

The kit is configured for fast and simple reaction setup, and reactions are assembled in a single tube, minimizing sample handling errors and expediting setup (Figure 1). Once the reactions are assembled, results are available in approximately 1 hour. The 25X RT-PCR Enzyme Mix is composed of Ambion's highly efficient ArrayScript™ Reverse Transcriptase, an MMLV RT capable of producing high cDNA yields, and AmpliTaq Gold® DNA Polymerase, the preferred hot-start DNA polymerase for specific target amplification. The 2X RT-PCR Buffer contains optimized reagents for efficient, robust reverse transcription and PCR. It also contains the passive reference dye, ROX™ Dye, for quantitative fluorescent signal normalization. Detection Enhancer is provided as an optional reagent for amplification of templates with high GC content or persistent secondary structure. Finally, a tube of ultrapure Nuclease-free Water is also provided.

Rigorous Linearity, Unsurpassed Sensitivity

To illustrate the consistent performance of the AgPath-ID™ One-Step RT-PCR Kit, serial dilutions of Virus A Control RNA containing 5 to 5 x 10^6 copies were amplified (Figure 2). The amplification plots show the consistent set of curves expected from highly efficient PCR, and the inset graph shows the reliability and efficiency of the PCR across a wide range of input template amounts.

Figure 3 shows amplification of a serial dilution of another control RNA, Virus B. Amounts of RNA were kept low (20 to 40,000 copies) in order to compare the analytical sensitivity of target amplification of the AgPath-ID™ kit and a competitor's RT-PCR reagent kit. The AgPath-ID™ One-Step RT-PCR Kit provided better analytical sensitivity than the competitor's kit across the dilution range. If your research demands consistent, reliable performance and maximum sensitivity, the AgPath-ID™ One-Step RT-PCR Kit delivers.

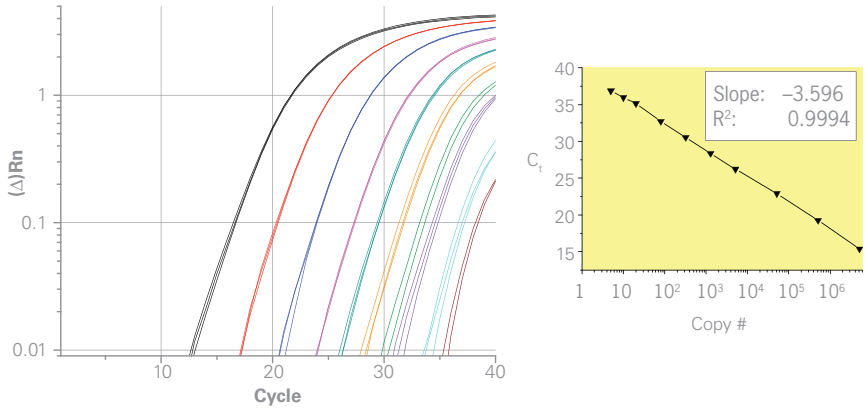

Figure 2. The AgPath-ID™ One-Step RT-PCR Kit Consistently Amplifies Across a Wide Dynamic Range of RNA Input. qRT-PCR targeting serially diluted Virus A Control RNA transcript (5 to 5 x 10^6 copies) demonstrates highly efficient and consistent performance. Reactions were performed on an Applied Biosystems 7500 Fast Real-Time PCR System.

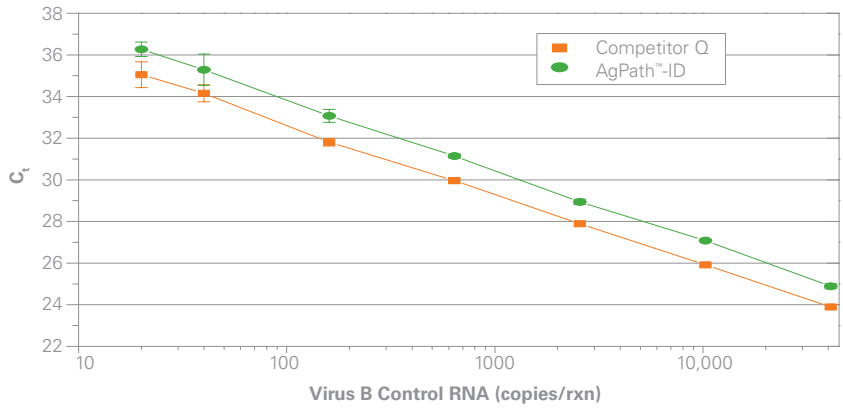

Figure 3. The AgPath-ID™ One-Step RT-PCR Kit Provides Higher Analytical Sensitivity Than a Competitor's Kit. Serially diluted Virus B Control RNA (20 to 40,000 copies) was amplified using the AgPath-ID™ One-Step RT-PCR Kit or a leading competitor's kit (Competitor Q). Reactions were performed on an Applied Biosystems 7500 Fast Real-Time PCR System. The AgPath-ID™ One-Step RT-PCR Kit provided earlier C_t values and better analytical sensitivity than the competitor's kit.

ORDERING INFORMATION

Description	Size	P/N
AgPath-ID™ One-Step RT-PCR Kit	100 rxns	AM1005
AgPath-ID™ One-Step RT-PCR Kit	500 rxns	4387424
AgPath-ID™ One-Step RT-PCR Kit	1000 rxns	4387391

Printed in the USA, 06/2011 Publication 137PB11-03

Applied Biosystems

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Phone 650.638.5800 | Toll Free 800.345.5224
www.appliedbiosystems.com

International Sales
For our office locations please call the division headquarters or refer to our Web site at
www.appliedbiosystems.com/about/offices.cfm

Certificate of Analysis

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AGPATH-ID(TM) ONE-STEP RT-PCR

Product No.	**AM1005**
Lot No.	**2309227**
Date of Manufacture	**12SEP2023**
Expiration Date	**11SEP2024**

TEST	SPECIFICATION	RESULT
NUCLEASE TESTS - ENZYME		
RNase activity		Pass
Exonuclease activity		Pass
Nonspecific endonuclease activity		Pass
FUNCTIONAL TESTS - ENZYME		
0 copies/reaction AIV input		Pass
40 copies/reaction AIV input	30.0 - 34.0 CT	Pass
80 copies/reaction AIV input	29.0 - 33.0 CT	Pass
160 copies/reaction AIV input	28.0 - 32.0 CT	Pass
320 copies/reaction AIV input	27.0 - 31.0 CT	Pass
1280 copies/reaction AIV input	25.5 - 29.5 CT	Pass
10240 copies/reaction AIV input	22.5 - 26.5 CT	Pass

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AGPATH-ID(TM) ONE-STEP RT-PCR

Product No.	**AM1005**
Lot No.	**2309227**
Date of Manufacture	**12SEP2023**
Expiration Date	**11SEP2024**

TEST	SPECIFICATION	RESULT
Xeno RNA	29.0 - 33.0 CT	Pass
NUCLEASE TESTS - BUFFER		
RNase activity		Pass
Exonuclease activity		Pass
Nonspecific endonuclease activity		Pass
FUNCTIONAL TESTS - BUFFER		
0 copies/reaction AIV input		Pass
40 copies/reaction AIV input	30.0 - 34.0 CT	Pass
80 copies/reaction AIV input	29.0 - 33.0 CT	Pass
160 copies/reaction AIV input	28.0 - 32.0 CT	Pass
320 copies/reaction AIV input	27.0 - 31.0 CT	Pass
1280 copies/reaction AIV input	25.5 - 29.5 CT	Pass

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AGPATH-ID(TM) ONE-STEP RT-PCR

Product No.	**AM1005**
Lot No.	**2309227**
Date of Manufacture	**12SEP2023**
Expiration Date	**11SEP2024**

TEST	SPECIFICATION	RESULT
10240 copies/reaction AIV input	22.5 - 26.5 CT	Pass
Xeno RNA	29.0 - 33.0 CT	Pass
NUCLEASE TESTS - WATER		
RNase activity		Pass
Exonuclease activity		Pass
Nonspecific endonuclease activity		Pass
FUNCTIONAL TESTS - WATER		
0 copies/reaction AIV input		Pass
40 copies/reaction AIV input	30 - 34 CT	Pass
80 copies/reaction AIV input	29 - 33 CT	Pass
160 copies/reaction AIV input	28 - 32 CT	Pass
320 copies/reaction AIV input	27 - 31 CT	Pass

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AGPATH-ID(TM) ONE-STEP RT-PCR

Product No.	**AM1005**
Lot No.	**2309227**
Date of Manufacture	**12SEP2023**
Expiration Date	**11SEP2024**

TEST	SPECIFICATION	RESULT
1280 copies/reaction AIV input	25.5 - 29.5 CT	Pass
10240 copies/reaction AIV input	22.5 - 26.5 CT	Pass
Xeno RNA	29 - 33 CT	Pass
FUNCTIONAL TESTS - ENHANCED TESTING		
500 copies/reaction of BVD RNA	31.2 - 34.2 CT	Pass
50 copies/reaction of BVD RNA	34.5 - 37.5 CT	Pass
No template control (NTC)	> = 40.0 CT	Pass

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Jazz Galaviz

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DÉCLARATION CE DE CONFORMITÉ · DICHIARAZIONE CE DI CONFORMITÀ

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(IVDD, Artikel 9 Absatz 1) nicht Teil der Liste A und B von Anhang II sein / (IVDD, Article9(1)) not be part of list A & B of annex II (IVDD, article 9, paragraphe 1) ne fait pas partie de la liste A et B de l'annexe II / (IVDD, articolo 9, paragrafo 1) non fanno parte dell'elenco A e B dell'allegato II

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meets the provisions of the directive 98/79/EC and its transpositions in national laws which apply to it. The declaration is valid in connection with the “final inspection report” of the device. /

remplit toutes les exigences de la directive sur les dispositifs médicaux 98/79/EC et de ses transpositions en droit national qui le concernent. La déclaration est valable si elle est associée au «rapport de l’inspection finale» du produit. /

soddisfa tutte le disposizioni della direttiva 98/79/EC e della loro trasposizione nel diritto nazionale che lo riguardano. Questa dichiarazione è valida in congiunzione con il “rapporto di ispezione finale” del prodotto.

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Registrier-Nr.: / Registration No.: / N°d’enregistrement: / Numero di registrazione:	
Benannte Stelle: / Notified Body: / Organisme notifié: / Organismo notificato:	

CE

Suzhou, 2022.01.01

General Manager

Ort, Datum / Place, date / Lieu, date / Luogo, data

Name und Funktion / Name and function / Nom et fonction / Nome e funzione

Pub. no. MAN0014479

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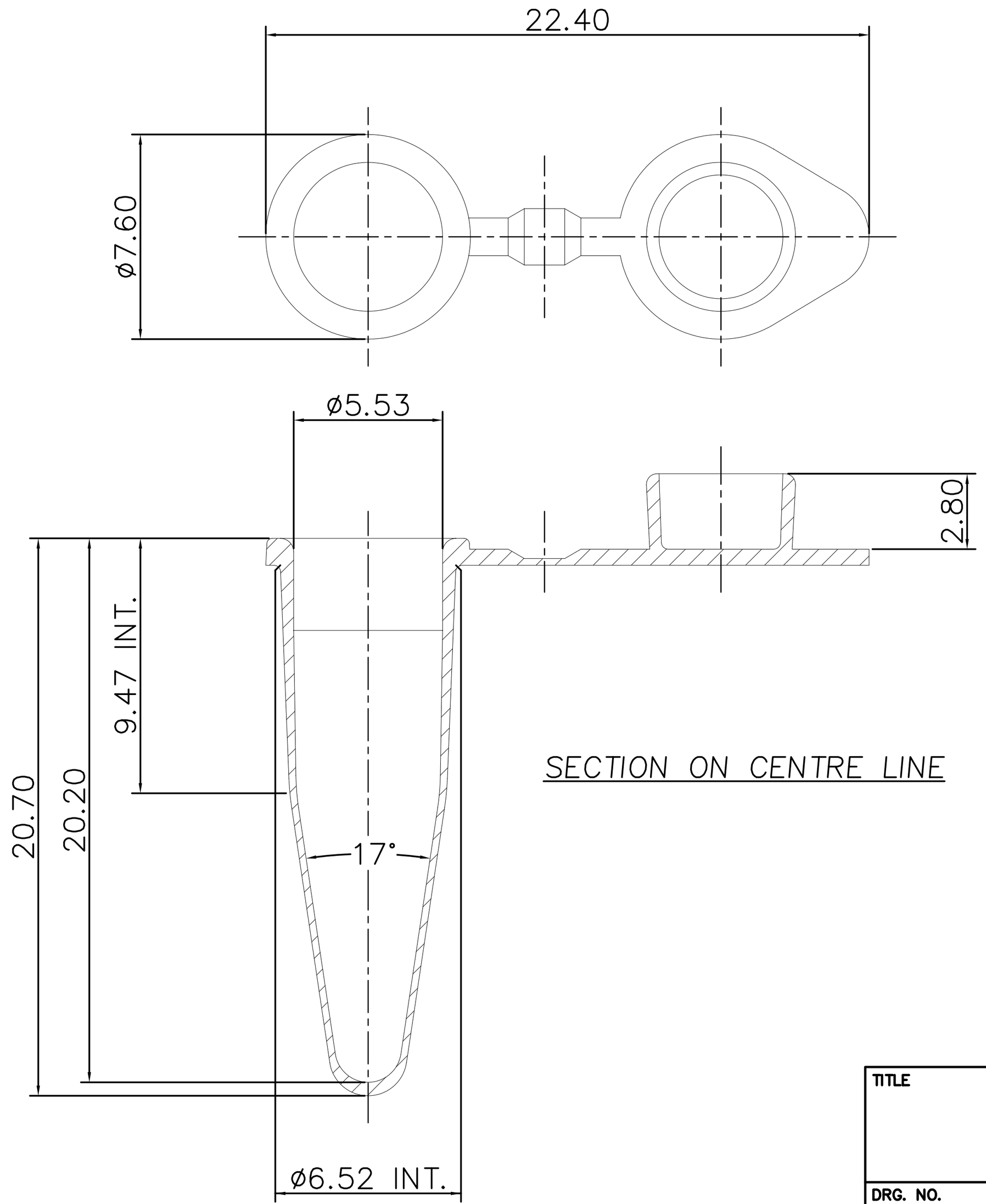

SECTION ON CENTRE LINE

All dimensions in mm

TITLE		
0.2ml Flat Cap Tube		
DRG. NO. AB–0620	ISSUE ORIGINAL	DATE 30/8/05

Certificate of Conformance

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OPTICAL 8 TUBE STRIP W/CAPS

Product No. **A30588**
Lot No. **1309738**

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Date: 2021-04-19 09:07-05:00

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Quality Assurance
Issued 19APR2021

MicroAmp™ plastic consumables compatibility chart for Applied Biosystems™ endpoint PCR systems and genetic analyzers

		3 x 32-well	96-well			96-well Fast	384-well		Genetic analyzers	
Product	**Cat. No.**	ProFlex™	Veriti™, ProFlex, SimpliAmp™	2720	9700	Veriti	ProFlex, Veriti	9700	310	3130, 3130xl, 3500, 3500xL, 3730, 3730xl
96-well 0.2 mL reaction plates										
Optical 96-Well Plate	N8010560, 4316813		●	●	●					●
Optical 96-Well Plate with Barcode	4306737, 4326659		●	●	●					●
96-Well Plate with Barcode & Optical Caps	403012		●	●	●					
Optical 96-Well Plate with Barcode & Optical Adhesive Films	4314320		●	●	●					
EnduraPlate Optical 96-Well Clear Plate with Barcode*	4483354, 4483352		●	●	●					●
TriFlex 3 x 32-Well Reaction Plate	A32810, A32811	●**	●	●	●					
96-well 0.1 mL reaction plates										
Fast Optical 96-Well Plate, 0.1 mL	4346907					●			●	●
Fast Optical 96-Well Plate with Barcode, 0.1 mL	4346906, 4366932					●			●	●
EnduraPlate Optical 96-Well Fast Clear Plate with Barcode*	4483485, 4483494					●			●	●
384-well reaction plates										
Optical 384-Well Plate	4343370						●	●		●
Optical 384-Well Plate with Barcode	4309849, 4326270, 4343814						●	●		●
EnduraPlate Optical 384-Well Clear Plate with Barcode*	4483285, 4483273						●	●		●
Strip tubes and caps										
Fast 8-Tube Strip, 0.1 mL	4358293					●				
Optical 8-Tube Strip with Attached Optical Caps, 0.2 mL	A30588	●	●	●	●					
8-Tube Strip with Attached Domed Caps, 0.2 mL	A30589	●	●	●	●					
8-Tube Strip, 0.2 mL*	N8010580	●	●	●	●				●	
Optical 8-Tube Strip, 0.2 mL	4316567	●	●	●	●					
8-Cap Strip*	N8010535, N8011535	●	●	●	●	●				
Optical 8-Cap Strip	4323032	●	●	●	●	●				
12-Cap Strip*	N8010534, N8011534		●	●	●	●				
Single tubes										
Fast Reaction Tube with Cap, 0.1 mL	4358297, 4358293					●				
Reaction Tube with Cap, 0.2 mL*	N8010540, N8010612, N8011540	●	●	●	●					
Reaction Tube without Cap, 0.2 mL*	N8010533, N8011533	●	●	●	●					
Optical Tube without Cap, 0.2 mL	N8010933	●	●	●	●					
Seals and covers										
Clear Adhesive Film	4306311		●		●	●	●	●		
Optical Adhesive Film	4360954, 4311971		●		●	●	●	●		
96-Well Full Plate Cover	N8010550			●	●					
32-Well Clear Adhesive Film	A32812	●**	●	●	●					
Accessories										
Splash-Free 96-Well Base	4312063		●	●	●	●				
96-Well Support Base	4379590		●	●	●	●				●
96-Well Base	N8010531		●	●	●					
96-Well Reaction Tube/Tray/Retainer Set, 0.2 mL	403083, 403086			●	●					

* Multiple colors are available. ** Do not use MicroAmp™ 3 x 32-Well Retainer (Cat. No. 4481669).

Note: Experiments using one or two 8-tube strips with attached caps require blank tube strips to balance lid pressure on the block or the use of the MicroAmp™ 96-Well Tray/Retainer Set (Cat. No. 4381850)—bottom part of tray *only*. For use with 96-well block of Applied Biosystems™ ProFlex, SimpliAmp, and Veriti thermal cyclers.

Find out more at **thermofisher.com/plastics**

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MicroAmp™ plastic consumables compatibility chart for Applied Biosystems™ real-time PCR systems

Product	Cat. No.	48-well	96-well			96-well Fast			384-well
		StepOne™	7000	7300, 7500	QuantStudio™ 3/5/6/7/12K, ViiA™ 7, 7900HT	StepOnePlus™	7500	QuantStudio 3/5/6/7/12K, ViiA 7, 7900HT	QuantStudio 5/6/7/12K, ViiA 7, 7900HT
96-well 0.2 mL reaction plates									
Optical 96-Well Plate	N8010560, 4316813		●	●	●				
Optical 96-Well Plate with Barcode	4306737, 4326659		●	●	●				
Optical 96-Well Plate with Barcode & Optical Caps	403012		●	●	●				
Optical 96-Well Plate with Barcode & Optical Adhesive Films	4314320		●	●	●				
EnduraPlate Optical 96-Well Clear Plate with Barcode*	4483354, 4483352			●**	●				
96-well 0.1 mL reaction plates									
Fast Optical 96-Well Plate, 0.1 mL	4346907					●	●	●	
Fast Optical 96-Well Plate with Barcode, 0.1 mL	4346906, 4366932					●	●	●	
EnduraPlate Optical 96-Well Fast Clear Plate with Barcode*	4483485, 4483494					●	●	●	
384-well reaction plates									
Optical 384-Well Plate	4343370								●
Optical 384-Well Plate with Barcode	4309849, 4326270, 4343814								●
EnduraPlate Optical 384-Well Clear Plate with Barcode*	4483285, 4483273								●
48-well reaction plates									
Fast Optical 48-Well Plate	4375816	●							
Strip tubes and caps									
Fast 8-Tube Strip, 0.1 mL	4358293	●				●	●	●	
Optical 8-Tube Strip with Attached Optical Caps, 0.2 mL	A30588		●	●	●				
Optical 8-Tube Strip, 0.2 mL	4316567		●	●	●				
Optical 8-Cap Strip	4323032	●	●	●	●	●	●	●	
Single tubes and caps									
Fast Reaction Tube with Cap, 0.1 mL	4358297	●				●		●	
Optical Tube without Cap, 0.2 mL	N8010933		●	●					
Seals and covers									
Optical Adhesive Film	4360954, 4311971		●	●	●	●	●	●	●
48-Well Optical Adhesive Film	4375323	●							
Reaction trays									
96-Well Tray/Retainer Set	403081		●						
Fast 48-Well Tray	4375282	●							
96-Well Tray for VeriFlex Blocks	4379983					●			
Accessories									
Splash-Free 96-Well Base	4312063		●	●	●	●	●	●	
96-Well Support Base	4379590		●	●	●	●	●	●	
96-Well Base	N8010531		●	●	●	●	●	●	

* Multiple colors are available. ** Requires use of proper adapter, Cat. No. A24820.

Note: Experiments using one or two 8-tube strips with attached caps require blank tube strips to balance lid pressure on the block or the use of the MicroAmp™ 96-Well Tray/Retainer Set (Cat. No. 4381850)—bottom part of tray *only*. For use with 96-well block of Applied Biosystems™ 7000, 7300, 7500, and ViiA 7 systems, and QuantStudio 3/5/6/7/12K instruments.

Find out more at **thermofisher.com/plastics**

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MicroAmp 8-Tube Strip with attached domed caps	✓	✓	✓	✓			

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Ordering information

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Second Edition

October 2010

TissueLyser Handbook

For high-throughput disruption of biological samples

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TissueLyser II*	
Catalog no.	**85300**
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* The TissueLyser II (cat. no. 85300) is an improved version of the TissueLyser (cat. no. 85200, 85210, or 85220; no longer available). All instructions and protocols in this handbook apply to both the TissueLyser II and the TissueLyser.

Storage

The TissueLyser should be stored upright in a dry environment at room temperature (15–25°C).

Product Use Limitations

The TissueLyser is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of many of the materials described in this text. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

Technical Assistance

At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the TissueLyser or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Introduction

Principle

The TissueLyser provides rapid and efficient disruption of up to 192 biological samples, including animal and human tissues, plant tissues, bacteria, and yeast. Disruption and homogenization are achieved through the beating and grinding effect of beads on the sample material as they are shaken together in the grinding vessels.

Disruption is critically important in order to release the nucleic acids from the sample material. Homogenization of the material acts to shear the high-molecular-weight cellular proteins and carbohydrates that may otherwise reduce binding of DNA and RNA to silica membranes or magnetic particles. Sample disruption using, for example, a mortar and pestle does not result in efficient homogenization. The TissueLyser both disrupts and homogenizes sample material in one simple and reliable step.

The TissueLyser is easily programmed to provide variable speeds from 3 to 30 Hz (180–1800 oscillations/minute) and run times from 10 seconds to 99 minutes.

Applications

The ability to process up to 192 samples per run makes the TissueLyser the ideal front-end solution to access biological information for genomics, transcriptomics, and proteomics applications. For next-generation high-throughput sequencing technologies such as polony sequencing, the TissueLyser is the disruption instrument of choice.

The TissueLyser enables fast and uniform disruption of animal and human tissues, plant tissues, bacteria, and yeast in various sample volumes in several formats. QIAGEN offers adapter sets for 2 x 96 collection microtubes (1.2 ml) or 2 x 24 microcentrifuge tubes (2 ml) as well as stainless steel and tungsten carbide beads. For disruption of large samples, grinding jar sets (10 ml) with stainless steel or Teflon® grinding balls are also available from QIAGEN. For more details about these and other accessories for the TissueLyser, see Appendix A (page 27).

The TissueLyser provides efficient disruption of biological material in each sample vessel for reproducible, high-quality results in downstream applications such as the purification of total DNA or RNA from a variety of human, animal, and plant tissues. A wide range of QIAGEN sample purification kits are compatible with the TissueLyser (see Tables 1–6, pages 7–10). Sample purification can be performed manually or can be automated using the QIAcube® , QIAsymphony™ SP, EZ1® Advanced, or BioRobot® or BioSprint® workstations. For more information about automated solutions from QIAGEN, see Appendix B (page 29).

This handbook provides guidelines on disrupting and homogenizing various sample materials for subsequent purification of DNA or RNA. Specific details on disruption and homogenization and nucleic acid purification, such as the amount of starting material and lysis buffer to use, can be found in the handbook supplied with each QIAGEN sample purification kit.

Table 1. Kits for RNA purification from animal or human tissues using spin columns

Sample type	Kit	Kit format	Page
Easy-to-lyse tissues (e.g., kidney, liver, and lung)	RNeasy® Micro Kit	Up to 5 mg tissue; automatable on QIAcube	16
	RNeasy Mini Kit	Up to 30 mg tissue; automatable on QIAcube	16
	RNeasy Protect Mini Kit	Up to 20 mg RNA*later*® stabilized tissue; automatable on QIAcube	16
	RNeasy Plus Micro Kit	Up to 5 mg tissue; includes gDNA Eliminator spin columns	16
	RNeasy Plus Mini Kit	Up to 30 mg tissue; includes gDNA Eliminator spin columns; automatable on QIAcube	16
Fiber-rich tissues (e.g., heart and muscle)	RNeasy Fibrous Tissue Mini Kit	Up to 30 mg tissue	16
	RNeasy Fibrous Tissue Midi Kit	Up to 250 mg tissue	16
Any type of tissue, including fatty tissues (e.g., adipose tissue and brain)	RNeasy Lipid Tissue Mini Kit	Up to 100 mg tissue; automatable on QIAcube	16
	miRNeasy Mini Kit	Up to 100 mg tissue; automatable on QIAcube	16

Table 2. Kits for RNA purification from animal or human tissues using magnetic particles or 96-well plates

Sample type	Kit	Kit format	Page
Easy-to-lyse tissues (e.g., kidney, liver, and lung)	EZ1 RNA Tissue Mini Kit	Magnetic particles; up to 10 mg tissue; automated on EZ1 Advanced* (1–6 samples per run)	16
	MagAttract® RNA Tissue Mini M48 Kit	Magnetic particles; up to 10 mg tissue; automated on BioRobot M48 (6–48 samples per run)	16
	QIAsymphony RNA Kit	Magnetic particles; up to 50 mg tissue; automated on QIAsymphony SP (1–96 samples per run)	16
Any type of tissue	EZ1 RNA Universal Tissue Kit	Magnetic particles; up to 50 mg tissue; automated on EZ1 Advanced* (1–6 samples per run)	16
	MagAttract RNA Universal Tissue M48 Kit	Magnetic particles; up to 50 mg tissue; automated on BioRobot M48 (6–48 samples per run)	16
	RNeasy 96 Universal Tissue Kits	96-well plate; up to 100 mg tissue; automatable on BioRobot Universal System (up to 80 mg tissue)†	16
	miRNeasy 96 Kit	96-well plate; up to 100 mg tissue	16

* Also automatable on BioRobot EZ1.

† Also automatable on BioRobot Gene Expression — Real-Time RT-PCR and BioRobot 8000.

Table 3. Kits for RNA purification from plant tissues, bacteria, and yeast

Sample type	Kit	Kit format	Page
Plant tissue (e.g., leaf)	RNeasy Plant Mini Kit	Spin column; up to 100 mg tissue; automatable on QIAcube	18
	RNeasy 96 Kit	96-well plate; up to 25 mg tissue	18
Bacteria (Gram-postive and -negative)	RNeasy Protect Bacteria Mini Kit	Spin column; up to 2.5 x 10^8 cells	20
	RNeasy Protect Bacteria Midi Kit	Spin column; up to 1.5 x 10^9 cells	20
Yeast	RNeasy Mini Kit	Spin column; up to 5 x 10^7 cells	21

Table 4. Kits for DNA purification from animal or human tissues

Kit	Kit format	Page
DNeasy® Blood & Tissue Kit	Spin column; up to 25 mg tissue; automatable on QIAcube	22
DNeasy 96 Blood & Tissue Kit	96-well plate; up to 20 mg tissue	22
QIAamp® DNA Mini Kit	Spin column; up to 25 mg tissue; automatable on QIAcube	22
EZ1 DNA Tissue Kit	Magnetic particles; up to 40 mg tissue; automated on EZ1 Advanced* (1–6 samples per run)	22
MagAttract DNA Mini M48 Kit	Magnetic particles; up to 40 mg tissue; automated on BioRobot M48 (6–48 samples per run)	22
QIAsymphony DNA Mini Kit	Magnetic particles; up to 50 mg tissue; automated on QIAsymphony SP (1–96 samples per run)	22

* Also automatable on BioRobot EZ1.

Table 5. Kits for DNA purification from plant tissues

Kit	Kit format	Page
DNeasy Plant Mini Kit	Spin column; up to 100 mg tissue; automatable on QIAcube	23
DNeasy Plant Maxi Kit	Spin column; up to 1 g tissue	25
DNeasy 96 Plant Kit	96-well plate; up to 50 mg tissue	23
MagAttract 96 DNA Plant Core Kit	Magnetic particles; up to 100 mg tissue; automatable on BioRobot Plant Science System — Genotyping*	23
BioSprint 15 DNA Plant Kit	Magnetic particles; up to 50 mg tissue; automated on BioSprint 15 (up to 15 samples per run)	23
BioSprint 96 DNA Plant Kit	Magnetic particles; up to 50 mg tissue; automated on BioSprint 96 (up to 96 samples per run)	23

* No longer available.

Table 6. Kits for simultaneous purification of multiple analytes from animal or human tissues

Analytes purified	Kit	Kit format	Page
DNA, RNA, and protein	AllPrep® DNA/RNA/ Protein Mini Kit	Spin column; up to 30 mg tissue	16
DNA and RNA	AllPrep DNA/RNA Micro Kit	Spin column; up to 5 mg tissue	16
	AllPrep DNA/RNA Mini Kit	Spin column; up to 30 mg tissue	16

QIAGEN Supplementary Protocols

Many of the protocols listed in this handbook are supplementary to the protocols found in the handbook of the specific kit being used. QIAGEN is constantly developing new protocols for existing products. These supplementary protocols can be obtained by contacting one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com) or visiting our Technical Support Center at www.qiagen.com/Support . Supplementary protocols can be identified by their reference number, which is made up of 2 letters followed by 2 numbers (e.g., RY23 — *Isolation of total RNA from plants using the RNeasy 96 Kit*).

Note: All protocols for use with the Mixer Mill can be used on the TissueLyser, without modification.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

For all protocols

- Kit for purification of DNA and/or RNA (see ordering information on pages 31–36 or visit www.qiagen.com)
- Optional: Reagent DX (see page 15 for details)
- Optional: Liquid nitrogen or dry ice (see the individual protocols)

Disruption of 2 x 48 samples

- TissueLyser Adapter Set 2 x 24*
- 2 ml microcentrifuge tubes (e.g., Eppendorf® Safe-Lock micro test tubes†)
- Stainless steel or tungsten carbide beads*
- Optional: TissueLyser Single-Bead Dispenser, 5 mm* or TissueLyser Single-Bead Dispenser, 7 mm*

Disruption of 2 x 96 samples

- TissueLyser Adapter Set 2 x 96*
- Collection Microtubes (racked)*
- Collection Microtube Caps*
- Optional: TissueLyser 3 mm Bead Dispenser, 96-Well* or TissueLyser 5 mm Bead Dispenser, 96-Well*

Disruption of 2 large samples

- For disruption of hard samples and disruption in liquid nitrogen: Grinding Jar Set, S. Steel*
- For disruption of most samples: Grinding Jar Set, Teflon*

* See page 31 for ordering information.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Important Notes

General remarks on disruption and homogenization

Efficient disruption and homogenization of the starting material is an absolute requirement for all nucleic acid purification procedures. Disruption and homogenization are 2 distinct steps:

- **Disruption**: Complete disruption of cell walls and plasma membranes of cells and organelles is absolutely required to release all the nucleic acids contained in the sample. Different samples require different methods to achieve complete disruption. Incomplete disruption results in significantly reduced DNA and RNA yields.
- **Homogenization**: Homogenization is necessary to reduce the viscosity of the cell lysates produced by disruption. Homogenization shears the high-molecular-weight cellular proteins and carbohydrates to create a homogeneous lysate. Incomplete homogenization results in inefficient binding of nucleic acids to QIAGEN silica membranes and magnetic particles and therefore significantly reduced DNA and RNA yields.

Cellular disruption is one of the most critical steps in nucleic acid purification. Disruption in lysis buffer alone, without physical shearing, may result in nucleic acid degradation by endogenous DNases and RNases. Incomplete disruption prevents the lysis buffer, which inactivates nucleases, from contacting nucleic acids within the intact cells. Furthermore, cellular debris that is not disrupted can result in decreased yield and increases the risk of clogging the purification column. After sample disruption, there should be no visible particulates (except when disrupting materials containing hard, noncellular components, such as connective tissue, bone, or woody plant tissue). QIAGEN kits and protocols contain recommendations for the most appropriate method of sample disruption and homogenization to maximize the yield and quality of your DNA and RNA preparation.

Disruption and homogenization using the TissueLyser

In bead-milling, cells and tissues can be disrupted by rapid agitation in the presence of beads. Disruption and simultaneous homogenization occur by the shearing and crushing action of the beads as they collide with the sample. Disruption efficiency is influenced by:

- Size and composition of beads
- Ratio of buffer to samples (if buffer is used)
- Amount of starting material
- Configuration of TissueLyser (i.e., speed and duration)
- Consistency of sample
- Type of disruption vessel

Disruption and homogenization methods

When using the TissueLyser in combination with QIAGEN sample purification kits, one of 2 methods for disruption and homogenization is carried out: samples are either disrupted and homogenized in lysis buffer at room temperature, or precooled and then disrupted and homogenized without lysis buffer. With the latter method, lysis buffer is added after disruption and homogenization.

The method of precooling samples depends on the TissueLyser accessory used. If using the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96, the adapter set should be stored at –80°C for at least 2 hours prior to starting disruption and homogenization, and the tubes containing the samples should be precooled on dry ice. If using a Grinding Jar Set, the jar containing the sample can be frozen in liquid nitrogen prior to starting disruption and homogenization.

Important: When using a TissueLyser Adapter Set, do not freeze the adapter set or the sample tubes in liquid nitrogen, as this may result in breakage of the tubes.

In special cases (e.g., the disruption of teeth or plant seeds), the sample can be disrupted and homogenized at room temperature without lysis buffer, although this increases the risk of nucleic acid degradation by nucleases.

Bead selection

For disruption of small samples, the optimal beads to use are 0.1–0.6 mm (mean diameter) glass beads for bacteria, 0.5 mm glass beads for yeast and unicellular animal cells, and 3–7 mm stainless steel or tungsten carbide beads for plant and animal/human tissues. It is essential that glass beads are pretreated before use by washing in concentrated nitric acid.* Pretreated (acid-washed) beads can be purchased from many vendors of biological supplies (e.g., Sigma, cat. nos. G1145, G1277, and G8772†). Disruption parameters for samples not addressed in this handbook must be determined empirically. For disruption of large samples, a Grinding Jar Set can be used, which is supplied with either stainless steel grinding balls (for disrupting hard samples such as bone or for disrupting samples in liquid nitrogen) or Teflon grinding balls (for disrupting most samples).

Note: Do not use Buffer RLT, Buffer RLT Plus, or QIAzol® Lysis Reagent in conjunction with tungsten carbide beads. These buffers react chemically with tungsten carbide, causing damage to the bead surface.

* When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

† This is not a complete list of suppliers and does not include many important vendors of biological supplies.

Operating the TissueLyser

The TissueLyser Adapter Set or Grinding Jar Set should be securely fixed into the clamps (arms) of the TissueLyser. For details, refer to the operating instructions supplied with the TissueLyser.

Disruption is carried out in high-speed (20–30 Hz) shaking steps. Disruption for 2 x 3 minutes at 20–30 Hz is usually sufficient to release RNA. If disrupting samples for subsequent DNA purification, disruption times should be shorter in order to prevent DNA shearing.

When using a TissueLyser Adapter Set, samples nearer to the TissueLyser move more slowly than samples further away from the TissueLyser. To ensure uniform disruption and homogenization, 2 shaking steps should be carried out. After the first shaking step, the TissueLyser Adapter Set should be disassembled and the rack of tubes should be rotated so that the tubes that were nearest to the TissueLyser are now outermost. The TissueLyser Adapter Set should then be reassembled before continuing with the second shaking step.

For optimal operation, the TissueLyser should always be balanced. A balance can be provided by assembling a second TissueLyser Adaptor Set with a rack of tubes containing only disruption beads, and fixing this adaptor set into the empty clamp. If using grinding jars, the balance should consist of a second grinding jar containing a grinding ball.

Disruption and homogenization in Buffer RLT Plus

RNeasy Plus Kits and certain AllPrep Kits are supplied with Buffer RLT Plus, a lysis buffer that provides optimal sample lysis as well as appropriate conditions for DNA binding to gDNA Eliminator columns or AllPrep DNA columns. When disrupting and homogenizing tissues in Buffer RLT Plus, excessive foaming may occur. This foaming is substantially reduced by adding Reagent DX to Buffer RLT Plus at a final concentration of 0.5% (v/v) before starting disruption and homogenization. Reagent DX has been carefully tested with RNeasy Plus Kits and AllPrep Kits, and has no effect on RNA purity or on downstream applications such as real-time RT-PCR. Buffer RLT Plus containing Reagent DX can be stored at room temperature (15–25°C) for at least 9 months. Reagent DX is supplied separately; for ordering information, see page 32.

Protocol: Purification of RNA or Multiple Analytes from Animal and Human Tissues

This protocol provides guidelines on disrupting animal and human tissues for purification of RNA or for simultaneous purification of DNA and RNA or DNA, RNA, and protein. If using a QIAGEN sample purification kit (see Tables 1, 2, and 6 on pages 7, 8, and 10), refer to the supplied handbook, which contains a complete protocol for sample disruption and purification.

Important points before starting

- Before beginning the procedure, read "Important Notes" (page 13).
- Ensure that you are familiar with operating the TissueLyser by referring to the operating instructions.
- If using a QIAGEN sample purification kit, read the supplied handbook carefully before starting.
- After storage in RNA*later* RNA Stabilization Reagent or Allprotect Tissue Reagent, tissues become slightly hard. If disrupting in Buffer RLT, we recommend increasing the volume of this buffer according to the protocols in the *RNeasy Mini Handbook*. In addition, the disruption time may be need to be extended.

Procedure

1. **Place the tissues in 2 ml microcentrifuge tubes or 1.2 ml collection microtubes containing 1 stainless steel bead (3–7 mm mean diameter).**

   If handling fresh or frozen tissue samples, keep the tubes on dry ice.

2. **Place the tubes at room temperature (15–25°C). Immediately add the appropriate volume of lysis buffer (e.g., Buffer RLT, Buffer RLT Plus, or QIAzol Lysis Reagent) to each tube.**

   Note: Do not use Buffer RLT, Buffer RLT Plus, or QIAzol Lysis Reagent with tungsten carbide beads, as these buffers can react with and damage the bead surface.

   Note: If using Buffer RLT Plus, we recommend adding Reagent DX to prevent excessive foaming. For details, see "Disruption and homogenization in Buffer RLT Plus" (page 15).

3. **Place the tubes in the TissueLyser Adapter Set 2 x 24 (if using 2 ml tubes) or the TissueLyser Adapter Set 2 x 96 (if using 1.2 ml tubes).**

4. **Operate the TissueLyser for 2 min at 20–30 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser are now outermost, and reassemble the adapter set. Operate the TissueLyser for another 2 min at 20–30 Hz.**

   The duration of disruption and homogenization depends on the tissue being processed and can be extended until no tissue debris is visible.

   Rearranging the tubes ensures uniform disruption and homogenization.

   If processing fiber-rich tissues, complete disruption and homogenization may sometimes not be possible. However, small amounts of debris have no effect on subsequent RNA purification with QIAGEN kits and are usually digested in the proteinase K step.

5. **Proceed with RNA, DNA/RNA, or DNA/RNA/protein purification.**

   Do not reuse the stainless steel beads.

Protocol: Purification of RNA from Plant Tissues

This protocol provides guidelines on disrupting plant tissues for subsequent RNA purification. If using a QIAGEN kit for RNA purification (see Table 3, page 9), refer to the supplied handbook, which contains a complete protocol for sample disruption and RNA purification.*

Important points before starting

- Before beginning the procedure, read "Important Notes" (page 13).
- Ensure that you are familiar with operating the TissueLyser by referring to the operating instructions.
- If using a QIAGEN kit for RNA purification, read the supplied handbook carefully before starting.
- Soft, fresh tissues from plants such as Nicotiana and Arabidopsis can often be disrupted and homogenized in lysis buffer. Hard tissues (e.g., woody plant materials) may require freezing and disruption under frozen conditions.

Procedure

1. **If handling frozen tissues, precool the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 by storing at –80°C for at least 2 h.**

   The adapter sets do not need to be precooled if handling fresh tissues.

2. **Place the tissues in 2 ml microcentrifuge tubes or 1.2 ml collection microtubes containing 1 stainless steel bead (3–7 mm mean diameter). If handling frozen tissues, keep the tubes on dry ice.**

   Note: Do not freeze the tubes in liquid nitrogen, as this may lead to breakage of the tubes.

3. **Immediately add the appropriate volume of lysis buffer (e.g., Buffer RLT or Buffer RLC) to each tube. If handling frozen tissues, do not add lysis buffer.**

   Note: Do not use Buffer RLT or Buffer RLC with tungsten carbide beads, as these buffers can react with and damage the bead surface.

4. **Place the tubes in the TissueLyser Adapter Set 2 x 24 (if using 2 ml tubes) or the TissueLyser Adapter Set 2 x 96 (if using 1.2 ml tubes).**

* If using the RNeasy 96 Kit, refer to supplementary protocol *Isolation of total RNA from plants using the RNeasy 96 Kit* (RY23).

5. **Operate the TissueLyser for 1 min at 30 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser are now outermost, and reassemble the adapter set. Operate the TissueLyser for another 1 min at 30 Hz.**

   The duration of disruption and homogenization depends on the tissue being processed and can be extended until no tissue debris is visible. If necessary, keep the samples on dry ice for several minutes in between the individual disruption steps to avoid thawing of the samples.

   Rearranging the tubes ensures uniform disruption and homogenization.

6. **Proceed with RNA purification. If frozen samples were disrupted, add lysis buffer, and proceed with RNA purification.**

   Do not reuse the stainless steel beads.

Protocol: Purification of RNA from Bacteria

This protocol provides guidelines on disrupting bacteria for subsequent RNA purification. If using an RNeasy Protect Bactera Kit for RNA purification (see Table 3, page 9), refer to the supplied *RNAprotect® Bacteria Reagent Handbook*, which contains complete protocols for sample disruption and RNA purification.

Important points before starting

- Before beginning the procedure, read "Important Notes" (page 13).
- Ensure that you are familiar with operating the TissueLyser by referring to the operating instructions.
- If using an RNeasy Protect Bacteria Kit for RNA purification, read the supplied handbook carefully before starting.
- Bead milling will disrupt most Gram-positive and Gram-negative bacteria, including mycobacteria. Gram-positive bacteria usually require more rigorous digestion (e.g., increased enzyme digestion time and temperature) and mechanical treatment than Gram-negative bacteria. For details, see the *RNAprotect Bacteria Reagent Handbook*.

Procedure

1. **Pellet the bacterial cells by centrifugation. Immediately add the appropriate volume of lysis buffer (e.g., Buffer RLT) to each sample and vortex vigorously.**
2. **Transfer each sample to 2 ml microcentrifuge tubes containing 25–50 mg acid-washed glass beads (150–600 µm mean diameter).**
3. **Place the tubes in the TissueLyser Adapter Set 2 x 24.**
4. **Operate the TissueLyser for 5 min at 30 Hz.**

   The duration of disruption and homogenization depends on the sample being processed and can be extended until no debris is visible.
5. **Proceed with RNA purification.**

Protocol: Purification of RNA from Yeast

This protocol provides guidelines on disrupting yeast cells for subsequent RNA purification. If using the RNeasy Mini Kit for RNA purification (see Table 3, page 9), refer to the supplied *RNeasy Mini Handbook*, which contains a complete protocol for sample disruption and RNA purification.

Important points before starting

- Before beginning the procedure, read "Important Notes" (page 13).
- Ensure that you are familiar with operating the TissueLyser by referring to the operating instructions.
- If using the RNeasy Mini Kit for RNA purification, read the supplied handbook carefully before starting.

Procedure

1. **Pellet the yeast cells by centrifugation. Immediately add the appropriate volume of lysis buffer (e.g., Buffer RLT) to each sample and vortex vigorously.**
2. **Transfer each sample to 2 ml microcentrifuge tubes containing 600 µl acid-washed glass beads (450–550 µm mean diameter).**
3. **Place the tubes in the TissueLyser Adapter Set 2 x 24.**
4. **Operate the TissueLyser for 5 min at 30 Hz.**

   The duration of disruption and homogenization depends on the sample being processed and can be extended until no debris is visible.
5. **Proceed with RNA purification.**

Protocol: Purification of DNA from Animal and Human Tissues

This protocol provides guidelines on disrupting animal and human tissues for subsequent DNA purification. If using a QIAGEN kit for DNA purification (see Table 4, page 9), refer to the following supplementary protocols for the complete procedure for sample disruption and DNA purification:

- **DNeasy Blood & Tissue Kit**: *Purification of total DNA from soft tissues using the TissueLyser and the DNeasy Blood & Tissue Kit* (DY11)
- **QIAamp DNA Mini Kit**: *Isolation of DNA from soft tissues using the TissueLyser and QIAamp DNA Mini Kit* (QA31)
- **EZ1 DNA Tissue Kit**: *Isolation of DNA from soft tissue using the TissueLyser and EZ1 DNA Tissue Kit* (MA23)
- **MagAttract DNA Mini M48 Kit**: *Isolation of DNA from soft tissue using the TissueLyser and MagAttract Mini M48 Kit* (MA22)

Important points before starting

- Before beginning the procedure, read "Important Notes" (page 13).
- Ensure that you are familiar with operating the TissueLyser by referring to the operating instructions.
- If using a QIAGEN kit for DNA purification, read the supplied handbook and appropriate supplementary protocol carefully before starting.

Procedure

1. **Place the tissues in 2 ml microcentrifuge tubes containing 1 stainless steel bead (5 mm mean diameter).**
2. **Add the appropriate volume of lysis buffer (e.g., Buffer ATL) to each tube.**
3. **Place the tubes in the TissueLyser Adapter Set 2 x 24.**
4. **Operate the TissueLyser for 20 s at 15 Hz.**

   Note: Exceeding this homogenization time and intensity may lead to significant fragmentation of genomic DNA.

   If working with fibrous tissues, cutting the tissue into smaller pieces before starting disruption will improve disruption efficiency.
5. **Proceed with DNA purification.**

Protocol: Purification of DNA from Plant Tissues (Mini Protocol)

This protocol provides guidelines on using TissueLyser Adapter Sets to disrupt plant tissues for subsequent DNA purification. If using a QIAGEN kit for DNA purification (see Table 5, page 10), refer to the supplied handbook, which contains a complete protocol for sample disruption and DNA purification.

Important points before starting

- Before beginning the procedure, read "Important Notes" (page 13).
- Ensure that you are familiar with operating the TissueLyser by referring to the operating instructions.
- If using a QIAGEN kit for DNA purification, read the supplied handbook carefully before starting.
- Fresh, frozen, or lyophilized tissues can be processed. Fresh tissues can be disrupted in lysis buffer at ambient temperature. Alternatively, fresh or frozen tissues can be disrupted without lysis buffer if they are precooled on dry ice and if the adapter sets are precooled at –80°C for at least 2 h. Lyophilized tissues can be disrupted without lysis buffer at ambient temperature. Disruption of tissues in lysis buffer yields DNA ideal for PCR, while disruption of tissues in liquid nitrogen yields DNA of a higher molecular weight. We do not recommend disrupting frozen tissues in lysis buffer as this results in low yields and degraded DNA.

Procedure

1. **If purifying DNA of higher molecular weight from fresh or frozen tissues, precool the TissueLyser Adapter Set 2 x 24 or TissueLyser Adapter Set 2 x 96 by storing at –80°C for at least 2 h.**

   The adapter sets do not need to be precooled if disrupting fresh tissues in lysis buffer or if disrupting lyophilized tissues.

2. **Place the tissues in 2 ml microcentrifuge tubes or 1.2 ml collection microtubes containing 1 tungsten carbide bead (3 mm mean diameter).**

3. **If purifying DNA of higher molecular weight from fresh or frozen tissues, precool the tubes by storing on dry ice.**

   Note: Do not freeze the tubes in liquid nitrogen, as this may lead to breakage of the tubes.

   The tubes do not need to be precooled if disrupting fresh tissues in lysis buffer or if disrupting lyophilized tissues.

4. **If necessary, add an appropriate volume of lysis buffer (e.g., Buffer AP1) to each tube.**

   Lysis buffer must not be added if disrupting precooled tissues or if disrupting lyophilized tissues.

5. **Place the tubes in the TissueLyser Adapter Set 2 x 24 (if using 2 ml tubes) or TissueLyser Adapter Set 2 x 96 (if using 1.2 ml tubes).**

6. **Operate the TissueLyser for 1 min at 25 Hz. Disassemble the adapter set, rotate the rack of tubes so that the tubes nearest to the TissueLyser are now outermost, and reassemble the adapter set. Operate the TissueLyser for another 1 min at 25 Hz.**

   Note: If processing precooled tissues, increasing the disruption time may lead to thawing and reduced DNA yield and quality.

7. **Add lysis buffer (e.g., Buffer AP1) if necessary, and proceed with DNA purification.**

   The tungsten carbide beads can be reused. For details on recovering and cleaning beads, refer to the *DNeasy Plant Handbook*.

Protocol: Purification of DNA from Plant Tissues (Maxi Protocol)

This protocol provides guidelines on using a Grinding Jar Set to disrupt plant tissues for subsequent DNA purification. If using the DNeasy Plant Maxi Kit for DNA purification (see Table 5, page 10), refer to the supplied *DNeasy Plant Handbook*, which contains a complete protocol for sample disruption and DNA purification.

Important points before starting

- Before beginning the procedure, read "Important Notes" (page 13).
- Ensure that you are familiar with operating the TissueLyser by referring to the operating instructions.
- If using the DNeasy Plant Maxi Kit for DNA purification, read the supplied handbook carefully before starting.
- Fresh, frozen, or lyophilized tissues can be processed. Fresh tissues can be disrupted in lysis buffer at ambient temperature. Alternatively, fresh or frozen tissues can be disrupted without lysis buffer if the jar containing the sample is frozen in liquid nitrogen. Lyophilized tissues can be disrupted without lysis buffer at ambient temperature. Disruption of tissues in lysis buffer yields DNA ideal for PCR, while disruption of tissues frozen in liquid nitrogen yields DNA of a higher molecular weight. We do not recommend disrupting frozen tissues in lysis buffer as this results in low yields and degraded DNA.

Procedure

1. **Place the tissues in 10 ml grinding jars containing 1 stainless steel grinding ball (20 mm mean diameter).**
2. **If purifying DNA of higher molecular weight from fresh or frozen tissues, freeze the jars in liquid nitrogen for 1 min.**

   The grinding jars do not need to be frozen if disrupting fresh tissues in lysis buffer or if disrupting lyophilized tissues.
3. **If necessary, add an appropriate volume of lysis buffer (e.g., Buffer AP1) to each jar.**

   Lysis buffer must not be added if processing frozen grinding jar sets or if disrupting lyophilized tissues.
4. **Operate the TissueLyser for 1 min at 30 Hz.**
5. **If purifying DNA of higher molecular weight from fresh or frozen tissues, freeze the jars in liquid nitrogen for 1 min.**

   The grinding jars do not need to be frozen if disrupting fresh tissues in lysis buffer or if disrupting lyophilized tissues.

6. **Operate the TissueLyser for 1 min at 30 Hz.**
7. **Add lysis buffer (e.g., Buffer AP1) if necessary, and proceed with DNA purification.**

   The stainless steel grinding balls can be reused. For details on recovering and cleaning grinding balls, refer to the *DNeasy Plant Handbook*.

Appendix A: TissueLyser Accessories

TissueLyser Adapter Set 2 x 24

This adapter set allows disruption of 48 (2 x 24) samples in parallel using standard 2 ml microcentrifuge tubes (e.g., Eppendorf Safe-Lock micro test tubes). Sample disruption can be carried out at room temperature or after storing the adapter set at –80°C for at least 2 hours. The adapter set can be cleaned with detergent, microbicides, or up to 96% ethanol. For more information, see the product sheet supplied with the TissueLyser Adapter Set.

TissueLyser Adapter Set 2 x 96

This adapter set allows disruption of 192 (2 x 96) samples in parallel using Collection Microtubes (racked). Sample disruption can be carried out at room temperature or after storing the adapter set at –80°C for at least 2 hours. The adapter set can be cleaned with detergent, microbicides, or up to 96% ethanol. For more information, see the product sheet supplied with the TissueLyser Adapter Set.

TissueLyser Single-Bead Dispenser, 5 mm

This bead dispenser dispenses individual beads (5 mm diameter) into any sample container. The reservoir holds approximately 150 beads. The TissueLyser Single-Bead Dispenser can be cleaned with water or detergent. For more information, see the product sheet supplied with the TissueLyser Single-Bead Dispenser.

TissueLyser Single-Bead Dispenser, 7 mm

This bead dispenser dispenses individual beads (7 mm diameter) into any sample container. The reservoir holds approximately 45 beads. The TissueLyser Single-Bead Dispenser can be cleaned with water or detergent. For more information, see the product sheet supplied with the TissueLyser Single-Bead Dispenser.

TissueLyser 3 mm Bead Dispenser, 96-well

This bead dispenser dispenses 96 beads (3 mm diameter) in parallel into Collection Microtubes (racked), enabling high-throughput disruption and homogenization. The reservoir holds approximately 1000 beads. The dispenser can be cleaned with water or detergent. For more information, see the product sheet supplied with the TissueLyser Bead Dispenser, 96-well.

TissueLyser 5 mm Bead Dispenser, 96-well

This bead dispenser dispenses 96 beads (5 mm diameter) in parallel into Collection Microtubes (racked), enabling high-throughput disruption and homogenization. The reservoir holds approximately 300 beads. The dispenser can be cleaned with water or detergent. For more information, see the product sheet supplied with the TissueLyser Bead Dispenser, 96-well.

Grinding Jar Set, S. Steel

The grinding jars allow disruption of 2 large samples in parallel using stainless steel grinding balls. Sample disruption can be carried out at room temperature or after freezing the grinding jars in liquid nitrogen. For more information, see the product sheet supplied with the Grinding Jar Set.

Grinding Jar Set, Teflon

The grinding jars allow disruption of 2 large samples in parallel using Teflon grinding balls. Sample disruption can be carried out at room temperature. For more information, see the product sheet supplied with the Grinding Jar Set.

Appendix B: Automated Solutions

Automated purification using QIAGEN spin-column kits

Purification of genomic DNA or total RNA from tissues can be fully automated on the QIAcube. The innovative QIAcube uses advanced technology to process QIAGEN spin columns, enabling seamless integration of automated, low-throughput sample prep into your laboratory workflow. Sample preparation using the QIAcube follows the same steps as the manual procedure (i.e., lyse, bind, wash, and elute), enabling you to continue using DNeasy Kits, QIAamp Kits, RNeasy Kits, and the miRNeasy Mini Kit for purification of high-quality DNA or RNA. For more information about the automated procedure, see the relevant protocol sheet available at www.qiagen.com/MyQIAcube .

The QIAcube.

The QIAcube is preinstalled with protocols for purification of plasmid DNA, genomic DNA, RNA, viral nucleic acids, and proteins, plus DNA and RNA cleanup. The range of protocols available is continually expanding, and additional QIAGEN protocols can be downloaded free of charge at www.qiagen.com/MyQIAcube .

Automated purification using magnetic particles and 96-well plates

Complete automated solutions from QIAGEN allow purification of genomic DNA or total RNA from human, animal, or plant tissues at a range of different throughputs using magnetic particles or 96-well plates (see Table 7, page 30). QIAGEN Instrument Service provides comprehensive support services to ensure the continued success of your automated applications. For more information about QIAGEN automation and QIAGEN Instrument Service, visit www.qiagen.com/automation .

Table 7. Automated purification of genomic DNA and total RNA from tissues

Workstation	Capability
EZ1 Advanced	Purification of genomic DNA or total RNA from 1–6 human samples per run
QIAsymphony SP	Purification of genomic DNA or total RNA from 1–96 animal or human samples per run
BioRobot Universal System	Purification of genomic DNA or total RNA in 96-well format from animal or human samples, plus downstream reaction setup
BioSprint 96	Purification of genomic DNA from up to 96 animal or plant samples per run

Low-throughput sample disruption

The TissueRuptor® is a handheld rotor–stator homogenizer that provides rapid and efficient disruption of individual samples for a wide range of downstream applications. The TissueRuptor uses transparent disposable probes, which helps to minimize the risk of cross-contamination and enables visual control of the sample disruption process. The TissueRuptor is an integral part of QIAGEN's complete solution for tissue management in gene expression, genotyping, and proteomics applications. Optimized protocols are available for sample disruption prior to manual or automated nucleic acid or protein purification, enabling a streamlined, efficient workflow. Purification of RNA, DNA, total nucleic acids, or protein can then be performed using QIAGEN kits. For more information about the TissueRuptor, visit www.qiagen.com/TissueRuptor .

Automated multicapillary gel electrophoresis

The revolutionary QIAxcel System enables fully automated and sensitive, high-resolution capillary electrophoresis for up to 96 samples per run. Ready-to-go gel cartridges reduce manual handling errors and eliminate the need for tedious gel preparation. With the QIAxcel System, analysis of DNA fragments, single- or multiplex PCR products, and qualitative and quantitative RNA analysis is now easier and faster than ever. To find out more, visit www.qiagen.com/QIAxcel .

Ordering Information

Product	Contents	Cat. no.
TissueLyser II	Universal laboratory mixer-mill disruptor, 100–120/220–240 V, 50/60 Hz	85300
Accessories		
TissueLyser Adapter Set 2 x 24	2 sets of Adapter Plates and 2 racks for use with 2 ml microcentrifuge tubes on the TissueLyser	69982
TissueLyser Adapter Set 2 x 96	2 sets of Adapter Plates for use with Collection Microtubes (racked) on the TissueLyser	69984
Grinding Jar Set, S. Steel (2 x 10 ml)	2 Grinding Jars (10 ml), 2 Stainless Steel Grinding Balls (20 mm)	69985
Grinding Jar Set, Teflon (2 x 10 ml)	2 Grinding Jars (10 ml), 2 Teflon Grinding Balls (20 mm)	69986
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with the TissueLyser system	69989
Tungsten Carbide Beads, 3 mm (200)	Tungsten Carbide Beads, suitable for use with the TissueLyser system	69997
TissueLyser Single-Bead Dispenser, 5 mm	For dispensing individual beads (5 mm diameter)	69965
TissueLyser Single-Bead Dispenser, 7 mm	For dispensing individual beads (7 mm diameter)	69967
TissueLyser 3 mm Bead Dispenser, 96-Well	For dispensing 96 beads (3 mm diameter) in parallel	69973
TissueLyser 5 mm Bead Dispenser, 96-Well	For dispensing 96 beads (5 mm diameter) in parallel	69975
Collection Microtubes (racked)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
Collection Microtube Caps	Nonsterile polypropylene caps for collection microtubes (1.2 ml) and round-well blocks, 960 in strips of 8	19566

Ordering Information

Product	Contents	Cat. no.
Related products		
RNeasy Kits — for purification of total RNA from cells, tissues, and yeast		
RNeasy Micro Kit (50)	For 50 preps: RNeasy MinElute® Spin Columns, Collection Tubes, DNase I, Carrier RNA, Buffers	74004
RNeasy Mini Kit (50)	For 50 preps: RNeasy Spin Columns, Collection Tubes, Buffers	74104
RNeasy Protect Kits — for stabilization and purification of total RNA from tissues		
RNeasy Protect Mini Kit (50)	For 50 preps: RNA*later* RNA Stabilization Reagent, RNeasy Spin Columns, Collection Tubes, Buffers	74124
RNeasy Plus Kits — for purification of total RNA from cells and tissues using gDNA Eliminator spin columns		
RNeasy Plus Micro Kit (50)	For 50 preps: RNeasy MinElute Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, Carrier RNA, Buffers	74034
RNeasy Plus Mini Kit (50)	For 50 preps: RNeasy Spin Columns, gDNA Eliminator Spin Columns, Collection Tubes, Buffers	74134
Reagent DX	1 ml Reagent DX in a screw-cap tube	19088
RNeasy Fibrous Tissue Kits — for purification of total RNA from fiber-rich tissues		
RNeasy Fibrous Tissue Mini Kit (50)	For 50 preps: RNeasy Spin Columns, Collection Tubes, Proteinase K, DNase I, Buffers	74704
RNeasy Fibrous Tissue Midi Kit (10)	For 10 preps: RNeasy Spin Columns, Collection Tubes, Proteinase K, DNase I, Buffers	75742
RNeasy Lipid Tissue Kits — for purification of total RNA from all types of tissue, including fatty tissues		
RNeasy Lipid Tissue Mini Kit (50)	For 50 preps: RNeasy Spin Columns, Collection Tubes, QIAzol Lysis Reagent, Buffers	74804

Ordering Information

Product	Contents	Cat. no.
RNeasy 96 Universal Tissue Kits — for purification of total RNA from all types of tissue in 96-well format		
RNeasy 96 Universal Tissue Kit (4)	For 4 x 96 preps: RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, Airpore Tape Sheets, QIAzol Lysis Reagent, Buffers	74881
RNeasy 96 Universal Tissue 8000 Kit (12)	For 12 x 96 preps on the BioRobot Universal System: RNeasy 96 Plates, Collection Microtubes, Elution Microtubes CL, Caps, S-Blocks, QIAzol Lysis Reagent, Buffers	967852
EZ1 RNA Tissue Mini Kit — for purification of total RNA from easy-to-lyse tissues on the BioRobot EZ1 workstation		
EZ1 RNA Tissue Mini Kit (48)	For 48 preps: Reagent Cartridges, Tips, Tip-Holders, Tubes, DNase I, Buffer RLT	959034
EZ1 RNA Universal Tissue Kit — for purification of total RNA from all types of tissue on the BioRobot EZ1 workstation		
EZ1 RNA Universal Tissue Kit (48)	For 48 preps: Reagent Cartridges, Tips, Tip-Holders, Tubes, QIAzol Lysis Reagent	956034
MagAttract RNA Tissue Mini M48 Kit — for purification of total RNA from easy-to-lyse tissues on the BioRobot M48 workstation		
MagAttract RNA Tissue Mini M48 Kit (192)	For 192 preps: MagAttract Suspension E, DNase I, Buffers	959236
MagAttract RNA Universal Tissue M48 Kit — for purification of total RNA from all types of tissue on the BioRobot M48 workstation		
MagAttract RNA Universal Tissue M48 Kit (192)	For 192 preps: MagAttract Suspension E, DNase I, QIAzol Lysis Reagent, Buffers	956336
QIAsymphony RNA Kit — for purification of total RNA from cells and tissues on the QIAsymphony SP		
QIAsymphony RNA Kit (192)	For 192 preps: 2 Reagent Cartridges, and Enzyme Racks	931636

Ordering Information

Product	Contents	Cat. no.
RNeasy Plant Mini Kit — for purification of total RNA from plants and fungi		
RNeasy Plant Mini Kit (20)	For 20 preps: RNeasy Spin Columns, QIAshredder Spin Columns, Collection Tubes, Buffers	74903
RNeasy 96 Kit — for purification of total RNA from cells in 96-well format		
RNeasy 96 Kit (4)	For 4 x 96 preps: RNeasy 96 Plates, Elution Microtubes CL, Caps, S-Blocks, Airpore Tape Sheets, Buffers	74181
RNeasy Protect Bacteria Kits — for stabilization and purification of total RNA from bacteria		
RNeasy Protect Bacteria Mini Kit (50)	For 50 preps: RNAprotect Bacteria Reagent, RNeasy Mini Kit	74524
RNeasy Protect Bacteria Midi Kit (10)	For 10 preps: RNAprotect Bacteria Reagent, RNeasy Midi Kit	75552
AllPrep DNA/RNA/Protein Mini Kit — for simultaneous purification of DNA, RNA, and protein from cells and tissues		
AllPrep DNA/RNA/ Protein Mini Kit (50)	For 50 preps: AllPrep DNA Spin Columns, RNeasy Spin Columns, Collection Tubes, Buffers	80004
AllPrep DNA/RNA Kits — for simultaneous purification of DNA and RNA from cells and tissues		
AllPrep DNA/RNA Micro Kit (50)	For 50 preps: AllPrep DNA Spin Columns, RNeasy MinElute Spin Columns, Collection Tubes, Carrier RNA, Buffers	80284
AllPrep DNA/RNA Mini Kit (50)	For 50 preps: AllPrep DNA Spin Columns, RNeasy Spin Columns, Collection Tubes, Buffers	80204
QIAamp DNA Mini Kit — for purification of genomic, mitochondrial, bacterial, parasite, or viral DNA		
QIAamp DNA Mini Kit (50)	For 50 preps: QIAamp Spin Columns, Collection Tubes, Proteinase K, Buffers	51304

Ordering Information

Product	Contents	Cat. no.
EZ1 DNA Tissue Kit — for automated purification of genomic DNA from 1–6 human samples on the BioRobot EZ1 workstation		
EZ1 DNA Tissue Kit (48)	For 48 preps: Reagent Cartridges, Tips, Tip-Holders, Tubes, Proteinase K, Buffer G2	953034
MagAttract DNA Mini M48 Kit — for automated purification of genomic DNA from 6–48 human samples on the BioRobot M48 workstation		
MagAttract DNA Mini M48 Kit (192)	For 192 preps: MagAttract Suspension B, Proteinase K, Buffers	953336
QIAsymphony DNA Kits — for purification of DNA from a wide range of sample types on the QIAsymphony SP		
QIAsymphony DNA Mini Kit (96)	For 96 preps of 400 µl each: 2 Reagent Cartridges, and Enzyme Racks	931235
QIAsymphony DNA Midi Kit (96)	For 96 preps of 1000 µl each: 2 Reagent Cartridges, and Enzyme Racks	931255
DNeasy Blood & Tissue Kit — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses		
DNeasy Blood & Tissue Kit (50)	For 50 preps: DNeasy Spin Columns, Collection Tubes, Proteinase K, Buffers	69504
DNeasy 96 Blood & Tissue Kit — for purification of total DNA from animal blood and tissues, and from cells, yeast, bacteria, or viruses in 96-well format		
DNeasy 96 Blood & Tissue Kit (4)	For 4 x 96 preps: DNeasy 96 Plates, Collection Microtubes, Caps, S-Blocks, Elution Microtubes RS, AirPore Tape Sheets, Proteinase K, Buffers	69581
DNeasy Plant Kits — for purification of total DNA from plants and fungi		
DNeasy Plant Mini Kit (50)	For 50 preps: DNeasy Spin Columns, QIAshredder Spin Columns, Collection Tubes, RNase A, Buffers	69104

Ordering Information

Product	Contents	Cat. no.
DNeasy Plant Maxi Kit (6)	For 6 preps: DNeasy Spin Columns, QIAshredder Spin Columns, Collection Tubes, RNase A, Buffers	68161
DNeasy 96 Plant Kit — for purification of total DNA from plants in 96-well format		
DNeasy 96 Plant Kit (6)	For 6 x 96 preps: DNeasy 96 Plates, Collection Microtubes, Caps, S-Blocks, Elution Microtubes RS, AirPore Tape Sheets, RNase A, Reagent DX, Buffers	69181
MagAttract 96 DNA Plant Core Kit — for manual or automated purification of total DNA from plants in 96-well format		
MagAttract 96 DNA Plant Core Kit (6)	For 6 x 96 preps: MagAttract Suspension A, RNase A, Buffers	67161
BioSprint 15 DNA Plant Kit — for automated purification of total DNA from plant tissue on the BioSprint 15 workstation		
BioSprint 15 DNA Plant Kit (60)	For 60 preps: MagAttract Suspension G, Rod Covers, Tube Strips, RNase A, Buffers	941514
BioSprint 96 DNA Plant Kit — for automated purification of total DNA from plant tissue on the BioSprint 96 workstation		
BioSprint 96 DNA Plant Kit (576)	For 576 preps: MagAttract Suspension G, Rod Covers, Microplates MP, S-Blocks, RNase A, Buffer RPW	941557

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

Notes

Notes

www.qiagen.com

Australia ▪ Orders 1-800-243-800 ▪ Fax 03-9840-9888 ▪ Technical 1-800-243-066

Austria ▪ Orders 0800-28-10-10 ▪ Fax 0800/28-10-19 ▪ Technical 0800-28-10-11

Belgium ▪ Orders 0800-79612 ▪ Fax 0800-79611 ▪ Technical 0800-79556

Brazil ▪ Orders 0800-557779 ▪ Fax 55-11-5079-4001 ▪ Technical 0800-557779

Canada ▪ Orders 800-572-9613 ▪ Fax 800-713-5951 ▪ Technical 800-DNA-PREP (800-362-7737)

China ▪ Orders 86-21-3865-3865 ▪ Fax 86-21-3865-3965 ▪ Technical 800-988-0325

Denmark ▪ Orders 80-885945 ▪ Fax 80-885944 ▪ Technical 80-885942

Finland ▪ Orders 0800-914416 ▪ Fax 0800-914415 ▪ Technical 0800-914413

France ▪ Orders 01-60-920-926 ▪ Fax 01-60-920-925 ▪ Technical 01-60-920-930 ▪ Offers 01-60-920-928

Germany ▪ Orders 02103-29-12000 ▪ Fax 02103-29-22000 ▪ Technical 02103-29-12400

Hong Kong ▪ Orders 800 933 965 ▪ Fax 800 930 439 ▪ Technical 800 930 425

Ireland ▪ Orders 1800 555 049 ▪ Fax 1800 555 048 ▪ Technical 1800 555 061

Italy ▪ Orders 800-789-544 ▪ Fax 02-334304-826 ▪ Technical 800-787980

Japan ▪ Telephone 03-6890-7300 ▪ Fax 03-5547-0818 ▪ Technical 03-6890-7300

Korea (South) ▪ Orders 080-000-7146 ▪ Fax 02-2626-5703 ▪ Technical 080-000-7145

Luxembourg ▪ Orders 8002-2076 ▪ Fax 8002-2073 ▪ Technical 8002-2067

Mexico ▪ Orders 01-800-7742-639 ▪ Fax 01-800-1122-330 ▪ Technical 01-800-7742-436

The Netherlands ▪ Orders 0800-0229592 ▪ Fax 0800-0229593 ▪ Technical 0800-0229602

Norway ▪ Orders 800-18859 ▪ Fax 800-18817 ▪ Technical 800-18712

Singapore ▪ Orders 1800-742-4362 ▪ Fax 65-6854-8184 ▪ Technical 1800-742-4368

Spain ▪ Orders 91-630-7050 ▪ Fax 91-630-5145 ▪ Technical 91-630-7050

Sweden ▪ Orders 020-790282 ▪ Fax 020-790582 ▪ Technical 020-798328

Switzerland ▪ Orders 055-254-22-11 ▪ Fax 055-254-22-13 ▪ Technical 055-254-22-12

UK ▪ Orders 01293-422-911 ▪ Fax 01293-422-922 ▪ Technical 01293-422-999

USA ▪ Orders 800-426-8157 ▪ Fax 800-718-2056 ▪ Technical 800-DNA-PREP (800-362-7737)

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1064945 10/2010

Sample & Assay Technologies

SAFETY DATA SHEET

Tungsten Carbide Beads 3mm

Version 1.0 Revision Date 11/26/2018 Print Date 08/17/2021

SECTION 1. IDENTIFICATION

Product name : Tungsten Carbide Beads 3mm

Manufacturer or supplier's details

Company : QIAGEN GmbH
QIAGEN Str. 1
D-40724 Hilden

Telephone : +49-02103-29-0

Responsible Department : QIAGEN Inc.
19300 Germantown Road
Germantown, MD 20874, USA
Tel.: 800-426-8157
http://support.qiagen.com

E-mail addressResponsible/issuing person : cpc@qiagen.com

Emergency telephone : CHEMTREC
USA & Canada 1-800-424-9300

Recommended use of the chemical and restrictions on use

Recommended use : Laboratory chemicals

SECTION 2. HAZARDS IDENTIFICATION

GHS Classification

Not a hazardous substance or mixture.

GHS Label element

Not a hazardous substance or mixture.

Other hazards

None known.

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

Substance / Mixture : Pure substance
Substance name : Tungsten Carbide Beads 3mm

Hazardous ingredients

SECTION 4. FIRST AID MEASURES

General advice : Show this material safety data sheet to the doctor in attendance.

If inhaled : Move to fresh air.
If symptoms persist, call a physician.

In case of skin contact	:	Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes. If symptoms persist, call a physician.
In case of eye contact	:	Remove contact lenses. Protect unharmed eye. Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
If swallowed	:	If accidentally swallowed obtain immediate medical attention. Rinse mouth with water. Never give anything by mouth to an unconscious person.
Most important symptoms and effects, both acute and delayed	:	No information available.
Notes to physician	:	No information available.

SECTION 5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	:	Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.
Specific hazards during fire fighting	:	Exposure to decomposition products may be a hazard to health.
Specific extinguishing methods	:	In the event of fire and/or explosion do not breathe fumes.
Special protective equipment for fire-fighters	:	Wear self-contained breathing apparatus for firefighting if necessary.

SECTION 6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures	:	Use personal protective equipment. Avoid dust formation. Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
Methods and materials for containment and cleaning up	:	Pick up and arrange disposal without creating dust. Keep in suitable, closed containers for disposal. Use mechanical handling equipment.

SECTION 7. HANDLING AND STORAGE

Advice on protection against fire and explosion	:	Provide appropriate exhaust ventilation at places where dust is formed.
Advice on safe handling	:	For personal protection see section 8. Smoking, eating and drinking should be prohibited in the application area.

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Ingredients with workplace control parameters

Personal protective equipment

Hand protection

Remarks	:	The choice of an appropriate glove does not only depend on

		its material but also on other quality features and is different from one producer to the other. Take note of the information given by the producer concerning permeability and break through times, and of special workplace conditions (mechanical strain, duration of contact).
Eye protection	:	Safety glasses
Skin and body protection	:	Choose body protection according to the amount and concentration of the dangerous substance at the work place. Footwear protecting against chemicals
Hygiene measures	:	Keep away from food and drink. When using do not eat, drink or smoke.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance	:	solid
Color	:	metallic
Odor	:	No data available
Odor Threshold	:	No data available
pH	:	No data available
Melting point/range	:	ca. 2,785 °C
Boiling point/boiling range	:	ca. 6,000 °C
Flash point	:	No data available
Evaporation rate	:	No data available
Burning rate	:	No data available
Upper explosion limit	:	No data available
Lower explosion limit	:	No data available
Vapor pressure	:	No data available
Relative vapor density	:	No data available
Relative density	:	No data available
Density	:	ca. 15.6 g/cm3
Solubility(ies)		
Water solubility	:	insoluble
Solubility in other solvents	:	insoluble
Partition coefficient: n-octanol/water	:	No data available
Autoignition temperature	:	No data available
Decomposition temperature	:	No data available
Viscosity		
Viscosity, dynamic	:	No data available
Viscosity, kinematic	:	No data available

Explosive properties	:	No data available
Oxidizing properties	:	No data available

SECTION 10. STABILITY AND REACTIVITY

Reactivity	:	No decomposition if stored and applied as directed.
Chemical stability	:	No decomposition if stored and applied as directed.
Possibility of hazardous reactions	:	Stable under recommended storage conditions. Hazardous decomposition products formed under fire conditions.
Conditions to avoid	:	No data available
Incompatible materials	:	No data available
Hazardous decomposition products	:	No decomposition if stored and applied as directed.

SECTION 11. TOXICOLOGICAL INFORMATION

Acute toxicity

Not classified based on available information.

Product:

Acute oral toxicity	:	No data available
Acute inhalation toxicity	:	No data available
Acute dermal toxicity	:	No data available

Skin corrosion/irritation

Not classified based on available information.

Product:

Remarks:
May cause skin irritation in susceptible persons.

Serious eye damage/eye irritation

Not classified based on available information.

Product:

Remarks:
May irritate eyes.

Respiratory or skin sensitization

Skin sensitization: Not classified based on available information.
Respiratory sensitization: Not classified based on available information.

Germ cell mutagenicity

Not classified based on available information.

Carcinogenicity

Not classified based on available information.

Reproductive toxicity

Not classified based on available information.

STOT-single exposure

Not classified based on available information.

STOT-repeated exposure

Not classified based on available information.

Aspiration toxicity

Not classified based on available information.

Further information

No data available

SECTION 12. ECOLOGICAL INFORMATION

Ecotoxicity

Product:

Toxicity to fish	:	No data available
Toxicity to algae	:	No data available
Toxicity to bacteria	:	No data available

Persistence and degradability

No data available

Bioaccumulative potential

Product:

Bioaccumulation	:	No data available

Mobility in soil

No data available

Other adverse effects

Product:

Additional ecological information	:	No data available

SECTION 13. DISPOSAL CONSIDERATIONS

Disposal methods

Contaminated packaging	:	Empty containers should be taken to an approved waste handling site for recycling or disposal.

SECTION 14. TRANSPORT INFORMATION

UNRTDG

Not regulated as a dangerous good

IATA-DGR

Not regulated as a dangerous good

IMDG-Code

Not regulated as a dangerous good

Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code

No data available

Domestic regulation

49 CFR
Not regulated as a dangerous good

49 CFR
Not regulated as a dangerous good

SECTION 15. REGULATORY INFORMATION

EPCRA - Emergency Planning and Community Right-to-Know

SARA 311/312 Hazards : No SARA Hazards

SECTION 16. OTHER INFORMATION

Full text of other abbreviations

(Q)SAR - (Quantitative) Structure Activity Relationship; ASTM - American Society for the Testing of Materials; bw - Body weight; DIN - Standard of the German Institute for Standardisation; ECx - Concentration associated with x% response; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ErCx - Concentration associated with x% growth rate response; GHS - Globally Harmonized System; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISO - International Organisation for Standardization; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; n.o.s. - Not Otherwise Specified; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; SADT - Self-Accelerating Decomposition Temperature; SDS - Safety Data Sheet; UN - United Nations; vPvB - Very Persistent and Very Bioaccumulative; DSL - Domestic Substances List (Canada); KECI - Korea Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); AICS - Australian Inventory of Chemical Substances; IECSC - Inventory of Existing Chemical Substances in China; ENCS - Existing and New Chemical Substances (Japan); ISHL - Industrial Safety and Health Law (Japan); PICCS - Philippines Inventory of Chemicals and Chemical Substances; NZIoC - New Zealand Inventory of Chemicals; TCSI - Taiwan Chemical Substance Inventory; CERCLA - Comprehensive Environmental Response, Compensation, and Liability Act; DOT - Department of Transportation; EHS - Extremely Hazardous Substance; HMIS - Hazardous Materials Identification System; MSHA - Mine Safety and Health Administration; NFPA - National Fire Protection Association; RCRA - Resource Conservation and Recovery Act; RQ - Reportable Quantity; SARA - Superfund Amendments and Reauthorization Act; CMR - Carcinogen, Mutagen or Reproductive Toxicant;

GLP - Good Laboratory Practice; ERG - Emergency Response Guide; NTP - National Toxicology Program; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods

Revision Date : 11/26/2018

The information provided in this Material Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

SAFETY DATA SHEET

Garnet/Glass/Ceramic/Stainless Steel Beads

Version	Revision Date:	Date of last issue: 08/09/2021
4.1	06/03/2023	Date of first issue: 03/08/2017

SECTION 1. IDENTIFICATION

Product name : Garnet/Glass/Ceramic/Stainless Steel Beads

Manufacturer or supplier's details

Company : QIAGEN GmbH
QIAGEN Str. 1
D-40724 Hilden

Telephone : +49-(0)2103-29-0

Responsible Department : QIAGEN Inc.
19300 Germantown Road
Germantown, MD 20874, USA
Tel.: 800-426-8157
http://support.qiagen.com

E-mail address
Responsible/issuing person : cpc@qiagen.com

Emergency telephone : CHEMTREC
USA & Canada 1-800-424-9300
CHEMTREC: 1-800-424-9300

Recommended use of the chemical and restrictions on use

Recommended use : Laboratory chemicals

SECTION 2. HAZARDS IDENTIFICATION

GHS classification in accordance with the OSHA Hazard Communication Standard (29 CFR 1910.1200)

Not a hazardous substance or mixture.

GHS label elements

Not a hazardous substance or mixture.

Other hazards

None known.

SECTION 3. COMPOSITION/INFORMATION ON INGREDIENTS

Substance / Mixture : Mixture

Components

No hazardous ingredients

SECTION 4. FIRST AID MEASURES

General advice : Show this material safety data sheet to the doctor in attendance.

If inhaled : Move to fresh air.

		If symptoms persist, call a physician.
In case of skin contact	:	Wash off immediately with soap and plenty of water while removing all contaminated clothes and shoes. If symptoms persist, call a physician.
In case of eye contact	:	Remove contact lenses. Protect unharmed eye. Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
If swallowed	:	If accidentally swallowed obtain immediate medical attention. Rinse mouth with water. Never give anything by mouth to an unconscious person.
Most important symptoms and effects, both acute and delayed	:	No information available.
Notes to physician	:	No information available.

SECTION 5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	:	Use extinguishing measures that are appropriate to local circumstances and the surrounding environment.
Specific hazards during fire fighting	:	Exposure to decomposition products may be a hazard to health.
Hazardous combustion products	:	No hazardous combustion products are known
Further information	:	In the event of fire and/or explosion do not breathe fumes.
Special protective equipment for fire-fighters	:	Wear self-contained breathing apparatus for firefighting if necessary.

SECTION 6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures	:	Use personal protective equipment. Avoid dust formation. Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
Environmental precautions	:	Do not allow uncontrolled discharge of product into the environment.
Methods and materials for containment and cleaning up	:	Keep in suitable, closed containers for disposal.

SECTION 7. HANDLING AND STORAGE

Advice on protection against fire and explosion	:	Normal measures for preventive fire protection.
Advice on safe handling	:	For personal protection see section 8. Smoking, eating and drinking should be prohibited in the application area.
Further information on storage stability	:	No decomposition if stored and applied as directed.

SECTION 8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Ingredients with workplace control parameters

Contains no substances with occupational exposure limit values.

Personal protective equipment

Hand protection

Remarks	:	The choice of an appropriate glove does not only depend on its material but also on other quality features and is different from one producer to the other. Take note of the information given by the producer concerning permeability and break through times, and of special workplace conditions (mechanical strain, duration of contact).
Eye protection	:	Safety glasses
Skin and body protection	:	Choose body protection according to the amount and concentration of the dangerous substance at the work place. Footwear protecting against chemicals
Hygiene measures	:	Keep away from food and drink. When using do not eat, drink or smoke.

SECTION 9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance	:	solid
Color	:	No data available
Odor	:	No data available
Odor Threshold	:	No data available
pH	:	No data available
Melting point/range	:	No data available
Boiling point/boiling range	:	No data available
Flash point	:	No data available
Evaporation rate	:	No data available
Burning rate	:	No data available
Upper explosion limit / Upper flammability limit	:	No data available
Lower explosion limit / Lower flammability limit	:	No data available
Vapor pressure	:	No data available
Relative vapor density	:	No data available
Relative density	:	No data available

Density	:	No data available
Solubility(ies)		
Water solubility	:	insoluble
Solubility in other solvents	:	No data available
Partition coefficient: n-octanol/water	:	No data available
Autoignition temperature	:	No data available
Decomposition temperature	:	No data available
Viscosity		
Viscosity, dynamic	:	No data available
Viscosity, kinematic	:	No data available
Explosive properties	:	No data available
Oxidizing properties	:	No data available

SECTION 10. STABILITY AND REACTIVITY

Reactivity	:	No decomposition if stored and applied as directed.
Chemical stability	:	No decomposition if stored and applied as directed.
Possibility of hazardous reactions	:	Stable under recommended storage conditions. Hazardous decomposition products formed under fire conditions.
Conditions to avoid	:	No data available
Incompatible materials	:	No data available
Hazardous decomposition products	:	No decomposition if stored and applied as directed.

SECTION 11. TOXICOLOGICAL INFORMATION

Acute toxicity

Not classified based on available information.

Product:

Acute oral toxicity	:	Remarks: No data available
Acute inhalation toxicity	:	Remarks: No data available
Acute dermal toxicity	:	Remarks: No data available

Skin corrosion/irritation

Not classified based on available information.

Product:

Remarks	:	May cause skin irritation in susceptible persons.

Serious eye damage/eye irritation

Not classified based on available information.

Product:

Remarks : May irritate eyes.

Respiratory or skin sensitization

Skin sensitization

Not classified based on available information.

Respiratory sensitization

Not classified based on available information.

Product:

Remarks : No data available

Germ cell mutagenicity

Not classified based on available information.

Carcinogenicity

Not classified based on available information.

IARC No ingredient of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

OSHA No component of this product present at levels greater than or equal to 0.1% is on OSHA's list of regulated carcinogens.

NTP No ingredient of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

Reproductive toxicity

Not classified based on available information.

STOT-single exposure

Not classified based on available information.

STOT-repeated exposure

Not classified based on available information.

Aspiration toxicity

Not classified based on available information.

Further information

Product:

Remarks : No data available

SECTION 12. ECOLOGICAL INFORMATION

Ecotoxicity

Product:

Toxicity to fish :

Remarks: No data available

Toxicity to algae/aquatic plants : Remarks: No data available

Toxicity to microorganisms : Remarks: No data available

Persistence and degradability

No data available

Bioaccumulative potential

Product:

Bioaccumulation : Remarks: No data available

Mobility in soil

No data available

Other adverse effects

Product:

Ozone-Depletion Potential : Regulation: 40 CFR Protection of Environment; Part 82 Protection of Stratospheric Ozone - CAA Section 602 Class I Substances
Remarks: This product neither contains, nor was manufactured with a Class I or Class II ODS as defined by the U.S. Clean Air Act Section 602 (40 CFR 82, Subpt. A, App.A + B).

Additional ecological information : No data available

SECTION 13. DISPOSAL CONSIDERATIONS

Disposal methods

Contaminated packaging : Empty containers should be taken to an approved waste handling site for recycling or disposal.

SECTION 14. TRANSPORT INFORMATION

International Regulations

UNRTDG
Not regulated as a dangerous good

IATA-DGR
Not regulated as a dangerous good

IMDG-Code
Not regulated as a dangerous good

Transport in bulk according to Annex II of MARPOL 73/78 and the IBC Code

Not applicable for product as supplied.

Domestic regulation

49 CFR
Not regulated as a dangerous good

Special precautions for user

Remarks : Not classified as dangerous in the meaning of transport regulations.

SECTION 15. REGULATORY INFORMATION

CERCLA Reportable Quantity
This material does not contain any components with a CERCLA RQ.

SARA 304 Extremely Hazardous Substances Reportable Quantity
This material does not contain any components with a section 304 EHS RQ.

SARA 302 Extremely Hazardous Substances Threshold Planning Quantity
This material does not contain any components with a section 302 EHS TPQ.

SARA 311/312 Hazards : No SARA Hazards

SARA 313 : This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

TSCA list
No substances are subject to a Significant New Use Rule.

No substances are subject to TSCA 12(b) export notification requirements.

SECTION 16. OTHER INFORMATION

Full text of other abbreviations

AIIC - Australian Inventory of Industrial Chemicals; ASTM - American Society for the Testing of Materials; bw - Body weight; CERCLA - Comprehensive Environmental Response, Compensation, and Liability Act; CMR - Carcinogen, Mutagen or Reproductive Toxicant; DIN - Standard of the German Institute for Standardisation; DOT - Department of Transportation; DSL - Domestic Substances List (Canada); ECx - Concentration associated with x% response; EHS - Extremely Hazardous Substance; ELx - Loading rate associated with x% response; EmS - Emergency Schedule; ENCS - Existing and New Chemical Substances (Japan); ErCx - Concentration associated with x% growth rate response; ERG - Emergency Response Guide; GHS - Globally Harmonized System; GLP - Good Laboratory Practice; HMIS - Hazardous Materials Identification System; IARC - International Agency for Research on Cancer; IATA - International Air Transport Association; IBC - International Code for the Construction and Equipment of Ships carrying Dangerous Chemicals in Bulk; IC50 - Half maximal inhibitory concentration; ICAO - International Civil Aviation Organization; IECSC - Inventory of Existing Chemical Substances in China; IMDG - International Maritime Dangerous Goods; IMO - International Maritime Organization; ISHL - Industrial Safety and Health Law (Japan); ISO - International Organisation for Standardization; KECI - Korea Existing Chemicals Inventory; LC50 - Lethal Concentration to 50 % of a test population; LD50 - Lethal Dose to 50% of a test population (Median Lethal Dose); MARPOL - International Convention for the Prevention of Pollution from Ships; MSHA - Mine Safety and Health Administration; n.o.s. - Not Otherwise Specified; NFPA - National Fire Protection

Association; NO(A)EC - No Observed (Adverse) Effect Concentration; NO(A)EL - No Observed (Adverse) Effect Level; NOELR - No Observable Effect Loading Rate; NTP - National Toxicology Program; NZIoC - New Zealand Inventory of Chemicals; OECD - Organization for Economic Co-operation and Development; OPPTS - Office of Chemical Safety and Pollution Prevention; PBT - Persistent, Bioaccumulative and Toxic substance; PICCS - Philippines Inventory of Chemicals and Chemical Substances; (Q)SAR - (Quantitative) Structure Activity Relationship; RCRA - Resource Conservation and Recovery Act; REACH - Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals; RQ - Reportable Quantity; SADT - Self-Accelerating Decomposition Temperature; SARA - Superfund Amendments and Reauthorization Act; SDS - Safety Data Sheet; TCSI - Taiwan Chemical Substance Inventory; TECI - Thailand Existing Chemicals Inventory; TSCA - Toxic Substances Control Act (United States); UN - United Nations; UNRTDG - United Nations Recommendations on the Transport of Dangerous Goods; vPvB - Very Persistent and Very Bioaccumulative

SDS Number : 600000008546

Revision Date : 06/03/2023

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