Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific GeneJET FFPE DNA Purification Kit #K0881, #K0882

Lot __

Expiry Date __

Read Storage information (p. 4) before the first use!

www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

The purity of isolated DNA from two sections (10 µm) of FFPE tissue is evaluated spectrophotometrically. The purified DNA has an A260/A280 ratio of 1.8–2.3. The functional quality of purified DNA is evaluated by PCR analysis.

Quality authorized by:  Jurgita Zilinskiene

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CONTENTS page

COMPONENTS OF THE KIT

GeneJET FFPE DNA Purification Kit	#K0881 50 preps	#K0882 250 preps
Proteinase K Solution	1.2 mL	4 × 1.3 mL
RNase A Solution	0.7 mL	3 × 1 mL
Digestion Buffer for GeneJET FFPE DNA Purification Kit	11 mL	55 mL
Binding Buffer for GeneJET FFPE DNA Purification Kit	11 mL	55 mL
Wash Buffer 1 (conc.) for GeneJET FFPE DNA Purification Kit	10 mL	40 mL
Wash Buffer 2 (conc.) for GeneJET FFPE DNA Purification Kit	10 mL	40 mL
Elution Buffer for GeneJET FFPE DNA Purification Kit	10 mL	40 mL
GeneJET DNA Purification Columns & Collection Tubes	50	250
Collection Tubes	50	250

STORAGE

The unopened vials of Proteinase K and RNase A solutions are stable at room temperature. Once the vial is opened, it should be stored at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).

DESCRIPTION

Formalin fixed and paraffin embedded (FFPE) tissue samples are routinely prepared from human surgical tissue samples. GeneJET DNA Purification Kit for FFPE samples is designed for fast and convenient purification of DNA from various amounts of FFPE tissue. Up to 8 sections (10 µm thickness) of FFPE samples can be used for genomic DNA extraction without overnight incubation. Elimination of toxic reagents commonly used for deparaffinization allows an environmentally-friendly procedure. Each preparation recovers up to 8 µg of genomic DNA from one section that can be eluted in 20 µL to 80 µL of Elution Buffer. High quality eluted DNA can be directly used in downstream applications such as qPCR, PCR, NGS library preparation, or stored at -20 °C.

PRINCIPLE

Sections of FFPE samples are subjected to enzymatic digestion and lysis to liberate genomic DNA. The released DNA is decrosslinked by heat incubation. Subsequently, the resulting solution is centrifuged and the supernatant containing DNA is mixed with Binding Buffer. After addition of ethanol, the lysate is loaded onto the purification column. The adsorbed DNA is washed to remove contaminants and then eluted with the Elution Buffer.

IMPORTANT NOTES

- DNA yield and quality from FFPE tissue may vary considerably depending on the tissue source, the thickness of the slice, the age of the sample, post-sampling delay before fixation, fixation time, etc.
- Paraffin sections can be stored at or below 4 °C for 1 year without observable effects on DNA yield and usability. Longer-term storage of FFPE sections may have negative effect on the DNA due to oxidation.
- To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples. For short–term, DNA solution may be stored at 0-4 °C, and for long-term at -20 °C.
- Add the indicated volume of ethanol (96-100%) to **Wash Buffer 1** (concentrated) and **Wash Buffer 2** (concentrated) prior to the first use:

	#K0881 50 preps		**#K0882** 250 preps	
	Wash Buffer 1	**Wash Buffer 2**	**Wash Buffer 1**	**Wash Buffer 2**
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96-100%)	**30 mL**	**30 mL**	**120 mL**	**120 mL**
Total volume:	40 mL	40 mL	160 mL	160 mL

After ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

- Check the **Digestion Buffer** and **Binding Buffer** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37 °C, then cool it back down to 25 °C before use.
- Set two thermal heating-blocks or waterbaths, one at 65 °C and one at 90 °C.
- It is recommended to use microcentrifuge tubes with screw caps in the steps 1 to 4 (see the Genomic DNA purification from FFPE samples protocol, p.6).
- Wear gloves when handling the **Binding Buffer, Wash Buffer 1 and Proteinase K Solution** as these reagents contain irritants (*see* p.8 for SAFETY INFORMATION).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipets and pipet tips
- Vortex mixer
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes (for sample lysis and DNA elution)
- Centrifuge for 1.5 mL microcentrifuge tubes (≥ 12,000 × g)
- Thermal heating-blocks or waterbath (adjustable to 65 °C and 90 °C)
- Disposable gloves

PROTOCOL OF GENOMIC DNA PURIFICATION FROM FFPE SAMPLES

- This protocol describes how to extract DNA from one to eight sections of FFPE tissue (when each section is up to 10 µm thick).

Step	Procedure
1	Add 200 µL of Digestion Buffer to a microcentrifuge tube (not provided) containing one or more sections (up to eight) of FFPE tissue. Incubate for 3 min at 90 °C. During the incubation mix the sample a few times by gently shaking the tube. Make sure the tissue sections stay submerged in the solution. After incubation, mix thoroughly with a vortex mixer to completely dissolve the paraffin. Cool the sample down to room temperature. If necessary, spin down briefly to clear the lid. **Note. It is not necessary to cut off the excess paraffin. Use a microcentrifuge tube with a screw cap. Incubation time should be prolonged to 6 min if more than one section of FFPE tissue is used.**
2	Add 20 µL of Proteinase K solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension. Spin down briefly to clear the lid.
3	Incubate the sample at 65 °C for 50 min in a thermoshaker or a water bath with occasional vortexing (300-400 rpm). **Note. Lysis time varies on the type and amount of FFPE sample processed. In some cases incubation time should be prolonged to 2 hours. Yield of DNA typically increases with extended lysis time**.
4	Transfer the samples to the heat block set to 90 °C and heat for 40 min. **Note. Prevent samples from being heated above 90 °C for a prolonged period of time.**
5	Centrifuge hot samples at 6000 × g for 1 min and transfer 200 µL of the digested lysate to a new 1.5 mL microcentrifuge tube (not provided). **Note. Transfer the entire liquid layer to a new tube leaving behind any wax particulates. Small amounts of debris will not affect the DNA yield. When using eight sections of FFPE tissue (each 10 µm thick), the digested lysate volume is 160-180 µL.**
6	Add 10 µL of RNase A solution and mix thoroughly by vortexing. Spin down briefly to clear the lid. Leave at room temperature for 10 min.
7	Add 200 µL of Binding Buffer. Vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.
8	Add 400 µL of ethanol (96-100%) to the sample. Vortex thoroughly for 10 seconds until homogeneous mixture is obtained. Spin down briefly to clear the lid.
9	Transfer the lysate to a GeneJET DNA Purification Column inserted into collection tube. Centrifuge for 1 min at 6000 × g. Discard the collection tube with the flow-through and place the column in a new collection tube (provided).
10	Add 500 µL of Wash Buffer 1 (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
11	Add 500 µL of Wash Buffer 2 (with ethanol added). Centrifuge for 3 min at maximum speed (≥ 12000 × g). Empty the collection tube, place the purification column back into the collection tube and re-spin the column for 1 min at maximum speed to dry the membrane. Discard the collection tube containing the flow-through solution and transfer the GeneJET DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not provided).

12	Add 60 µL of Elution Buffer directly to the center of the purification column membrane. Leave for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note. For maximum DNA yield, repeat the elution step with additional 60 µL of Elution Buffer. (perform the second elution using different tube).** **If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., one section of FFPE sample) the volume of the Elution Buffer added to the column can be reduced to 20 µL. Elution volumes in the range of 20-80 µL are recommended, the default volume is 60 µL.**
13	Discard the column. Use the purified DNA immediately in downstream applications or store at -20 °C.

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more tissue than indicated in lysis protocols. **Starting material was not completely digested.** If the suspension does not clarify during Proteinase K digestion, this could indicate that it is oxidized. Extend the Proteinase K digestion at 65 °C until complete lysis occurs and no particles remain. **Ethanol was not added to the lysate.** Make sure that the ethanol was added to the lysate before applying the sample to the Purification Column. **Ethanol was not mixed with the lysate.** After the addition of ethanol to the lysate mix the sample by vortexing or pipetting. **Ethanol was not added to Wash Buffers.** Make sure that ethanol was added to Wash Buffer 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on p.5. **Poor sample quality.** Sample fixation, embedding and storage have a significant impact on quality and amount of the DNA in FFPE tissue samples.
RNA contamination	**RNase A treatment was not carried out.** Carry out RNase A treatment step described in the purification procedure.
Column becomes clogged during purification	**Excess sample was used during lysate preparation.** Too much starting material was used. Overloading may lead to a decrease in DNA yield. **Tissue was not completely digested.** Insufficient disruption and / or homogenization of starting material. Extend the Proteinase K digestion at 65 °C until complete lysis occurs and no particles remain.
Inhibition of downstream enzymatic reactions	**Purified DNA contains residual ethanol.** Do not let the flow-through touch the column outlet after the second wash with Wash Buffer 2. Always re-spin the column for an additional 1 min. at maximum speed (≥ 12000 × g) after the second wash. **Purified DNA contains residual salt.** Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer 1 first and then proceed to wash with Wash Buffer 2.

SAFETY INFORMATION

Proteinase K

Danger
Hazard statements:
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
Precautionary statements:
P285 In case of inadequate ventilation wear respiratory protection.
P261 Avoid breathing dust/fume/gas/mist/vapours/spray.
P342+P311 If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
P304+P341 IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Binding Buffer

Warning
Hazard statements:
H302 Harmful if swallowed.
H315 Causes skin irritation
H319 Causes serious eye irritation.
Precautionary statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P321 Specific treatment (see on this label).
P362 Take off contaminated clothing and wash before reuse.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

Wash Buffer 1

Warning
Hazard statements:
H302 Harmful if swallowed.
H315 Causes skin irritation
H319 Causes serious eye irritation.
Precautionary statements:
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P321 Specific treatment (see on this label).
P362 Take off contaminated clothing and wash before reuse.
P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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CERTIFICATE OF ANALYSIS

K0881 GeneJET FFPE DNA Purification Kit

Packaging Lot: **2702325**
Expiry Date: 22.12.2024 (DD.MM.YYYY)
Storage: at 5±3°C
Note: IMPORTANT Check Individual Components for Storage Conditions

Filling lots for components in package:

Lot	Quantity	Description
2658536	1.2 mL	Proteinase K Solution, 20mg/ml
2631028	0.7 mL	RNase A Solution, 10mg/ml
2640197	10 mL	Elution Buffer
2685150	11 mL	Digestion Buffer
2644944	11 mL	Lysis Solution
01326857	10 mL	Wash Buffer I (concentrated)
2644841	10 mL	Wash Buffer II (concentrated)
2689585	1 pack	GeneJET DNA Purification Columns & collection Tubes
2684497	1 pack	Collection Tubes 2 ml

QUALITY CONTROL

Parameter	Method	Requirement	Result
Specific activity (RNase A)	One unit is the amount of the enzyme which produces an increase in soluble reaction products by an OD of 1.0 at A260 nm using yeast RNA as substrate in 15 minutes at 37 °C.	≥ 5000 U/mg	Conforms
Activity (Proteinase K)	The unit activity of a solution of Proteinase K is determined. One unit liberates 1 µmol of Folin-positive amino acids, measured as tyrosine, at 37°C, pH 7.5, using denatured bovine hemoglobin as the substrate.	Within range of predetermined specifications	Conforms
pH (Relevant kit components)	Measured using a pH meter.	Within range of predetermined specifications	Conforms
Density (Relevant kit components)	Measured using a densitometer.	Within range of predetermined specifications	Conforms
Refractive Index (Relevant kit components)	Measured using a refractometer.	Within range of predetermined specifications	Conforms
Conductivity (Relevant kit components)	Measured using a conductometer.	Within range of predetermined specifications	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė 

TopVision Agarose

Catalog Number R0491, R0492

Pub. No. MAN0013146 **Rev.** C.00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support..**

Contents and storage

Cat. No.	Contents	Amount	Storage
R0491	TopVision Agarose	100 g	15 °C to 25 °C
R0492		500 g	

Description

Thermo Scientific TopVision Agarose is highly purified agarose with very low EEO values certified by strict quality control test procedures.

Characteristics

Electroendosmosis EEO, -M_r	0.08 - 0.11
Gel strength (1 % gel)	≥1200 g/cm^2
Gel strength (1.5 % gel)	≥2300 g/cm^2
Gel point (1.5 % gel)	34.0 - 38.0 °C
Moisture	≤8.0 %
DNA binding	none detected
Ash	≤0.50 %
Sulphate	≤0.10 %

Application

- Preparation of analytical gels for sharp resolution of nucleic acid fragments.
- Preparation of DNA preparative gels in routine molecular biology techniques.
- Suitable for blotting assays.

Note

- May be used at concentrations between 0.4 - 5 % with all typical buffer systems.
- The DNA can be rapidly recovered from agarose gels after electrophoresis using the Thermo Scientific GeneJET Gel Extraction Kit (#K0691, *see* www.thermoscientific.com) and is compatible with all common downstream applications including restriction, ligation, etc.

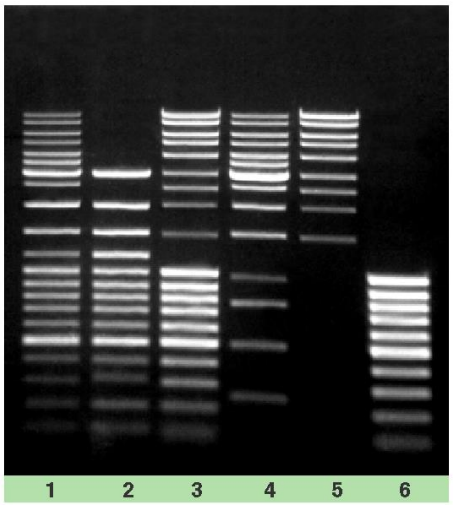

Fig. 1. Electrophoresis of DNA Size Markers.

1 % TopVision™ Agarose, 1X TAE buffer.
1 –GeneRuler DNA Ladder Mix
2 – GeneRuler 100 bp Plus DNA Ladder
3 – GeneRuler DNA Ladder Mix, ready-to-use
4 – GeneRuler 1 kb DNA Ladder
5 –MassRuler High Range DNA Ladder, ready-to-use
6 – GeneRuler 100 bp DNA Ladder

Limited product warranty

Life Technologies Corporation and/or it 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**.

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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.

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CERTIFICATE OF ANALYSIS

R0492 **TopVision Agarose**
Packaging Lot: **2703081**
Expiry Date: 30.04.2027 (DD.MM.YYYY)
Storage: at room temperature

Filling lots for components in package:

Lot	Quantity	Description
2702996	500 g	TopVision Agarose, 500g

QUALITY CONTROL

Parameter	Method	Requirement	Result
Ribonucleases	No detectable RNase activity.	Not detectable	Conforms
Endonucleases	No detectable DNase activity.	Not detectable	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė

Thermo SCIENTIFIC

PRODUCT INFORMATION

PCR Master Mix (2X)

#K0171 for 200 rxns

Lot: __ Expiry Date: __

Store at -20°C

Ordering Information

Component	#K0171 200 rxns of 50 µL	#K0172 1000 rxns of 50 µL
PCR Master Mix (2X)	4 × 1.25 mL	20 × 1.25 mL
Water, nuclease-free	4 × 1.25 mL	20 × 1.25 mL

www.thermoscientific.com/onebio

Rev.11

Description

PCR Master Mix is a 2X concentrated solution of *Taq* DNA polymerase, dNTPs and all other components required for PCR, except DNA template and primers. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. The mix is optimized for efficient and reproducible PCR.

Applications

- High throughput PCR.
- Routine PCR with high reproducibility.
- Generation of PCR products for TA. cloning.
- RT-PCR.

Composition of the PCR Master Mix (2X)

0.05 U/µL *Taq* DNA polymerase, reaction buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP).

PROTOCOL

1. Gently vortex and briefly centrifuge PCR Master Mix (2X) after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 µL reaction:

PCR Master Mix (2X)	25 µL
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	10 pg - 1 µg
Water, nuclease-free	to 50 µL
Total volume	50 µL

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	25-40
Annealing	Tm-5	30 s	
Extension	72	1 min/kb	
Final Extension	72	5-15 min	1

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform "no template control" (NTC) reactions to check for contamination.

GUIDELINES FOR PRIMER DESIGN

Use the Thermo Scientific REviewer primer design software at www.thermoscientific.com/reviewer or follow the general recommendations for PCR primer design as outlined below:

- PCR primers are generally 15-30 nucleotides long.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3'-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3'-end.
- When introducing restriction enzyme sites into primers, refer to the table "Cleavage efficiency close to the termini of PCR fragments" located on www.thermoscientific.com/onebio to determine the number of extra bases required for efficient cleavage.

Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:

$$Tm = 4 (G + C) + 2 (A + T),$$

where G, C, A, T represent the number of respective nucleotides in the primer.

If the primer contains more than 25 nucleotides we recommend using specialized computer programs e.g., REviewer™ (www.thermoscientific.com/reviewer) to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amounts of template DNA for a 50 µL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

All routine DNA purification methods can be used to prepare the template, e.g. Thermo Scientific GeneJET Genomic DNA Purification Kit (#K0721) or GeneJET™ Plasmid Miniprep Kit (#K0502). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, may inhibit DNA polymerase. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually removes trace contaminants from DNA samples.

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.

For degenerate primers and primers used for long PCR we recommend higher primer concentrations in the range of 0.3-1 µM.

(continued on reverse page)

CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95°C is sufficient.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

Primer annealing

The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.

Extension

The optimal extension temperature for *Taq* DNA polymerase is 70-75°C. The recommended extension step is 1 min/kb at 72° for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 1 min/kb.

Number of cycles

If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for an additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product has to be cloned into a TA vector, e.g. using Thermo Scientific InsTAclone PCR Cloning Kit (#K1213), the final extension step may be prolonged to 30 min to ensure the highest efficiency of 3'-dA tailing of the PCR product. If the PCR product has to be used for cloning using Thermo Scientific CloneJET PCR Cloning Kit (#K1231), the final extension step can be omitted.

Troubleshooting

For troubleshooting please visit www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation of DNA was observed after incubation of 1 µg of pUC19 DNA with 25 µL of PCR Master Mix (2X) in 50 µL of reaction mixture for 4 hours at 37°C and at 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of DNA was observed after incubation of 1 µg of lambda DNA/HindIII fragments with 25 µL of PCR Master Mix (2X) in 50 µL of reaction mixture for 4 hours at 37°C and at 70°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 1 µg of [^{3}H]-RNA with 25 µL PCR Master Mix (2X) in 50 µL of reaction mixture for 4 hours at 37°C and at 70°C.

Functional Assay

PCR Master Mix (2X) was tested for amplification of 956 bp single copy gene from human genomic DNA.

Quality authorized by:  Jurgita Zilinskiene

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CERTIFICATE OF ANALYSIS

K0171 **PCR Master Mix (2X)**
Packaging Lot: **2681185**
Expiry Date: 28.02.2025 (DD.MM.YYYY)
Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
2654392	4 x 1.25 mL	2X PCR Master Mix
2652017	4 x 1.25 mL	Water, Nuclease-free

QUALITY CONTROL

Parameter	Method	Requirement	Result
Endodeoxyribonucleases (nicking activity)	Incubation of supercoiled plasmid DNA with PCR Master Mix (2X).	Not detectable	Conforms
Exodeoxyribonuclease	Incubation of Lambda DNA/HindIII fragments with PCR Master Mix (2X).	Not detectable	Conforms
Ribonucleases	Incubation of [3H]-RNA with PCR Master Mix.	Not detectable	Conforms
Functional testing	PCR amplification of 956 bp single copy gene from human genomic DNA and analysis on agarose gel.	Reaction produces specific PCR product	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė

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PRODUCT INFORMATION

Thermo Scientific
GeneJET Whole Blood RNA Purification Mini Kit
#K0761

www.thermoscientific.com/onebio

#K0761
Lot __
Exp. __

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Whole Blood RNA Purification Mini Kit is qualified by isolating total RNA from 500 µL of human whole blood following the protocol outlined in the manual. The quality of isolated RNA is evaluated spectrophotometrically and by agarose gel electrophoresis. The purified RNA has an $A_{260/280}$ ratio between 1.9 and 2.1 and the RNA integrity number (RIN) of ≥8.

Quality authorized by: Jurgita Žilinskienė

Rev.3 .

CONTENTS page

COMPONENTS OF THE KIT

GeneJET Whole Blood RNA Purification Mini Kit	#K0761 50 preps
Lysis Buffer	40 mL
Wash Buffer WB 1 (concentrated)	40 mL
Wash Buffer 2 (concentrated)	23 mL
Water, nuclease-free	30 mL
GeneJET RNA Purification Columns pre-assembled with Collection Tubes	50
Collection Tubes, 2 mL	50
Collection Tubes, 1.5 mL	50

STORAGE

All kit components should be stored at room temperature (15-25°C).
Note. Close the bag with GeneJET RNA Purification Columns tightly after each use!

DESCRIPTION

The GeneJET™ Whole Blood RNA Purification Mini Kit is designed for rapid and efficient purification of high quality total RNA from whole blood and related body fluids. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The standard procedure takes less than 15 minutes following cell lysis. The purified high quality RNA can be used in a wide range of downstream applications such as RT-PCR, RT-qPCR, Northern blotting and other RNA-based analysis.

PRINCIPLE

Blood is collected into vials and stabilized by anticoagulants. Cells are lysed in a buffer containing guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogenous RNases. The lysate is then mixed with ethanol and loaded on the purification column. The chaotropic salt and ethanol facilitate RNA binding to the silica membrane when the lysate is spun through the column. Subsequently, impurities are effectively removed by treating the column with the provided wash buffers. Pure RNA is then eluted under low ionic strength conditions with the provided nuclease-free water.

Table 1. Typical total RNA yields from various sources.

Source	Amount	Yield, µg
Human blood	500 µL	1.2-1.8
Mouse blood	500 µL	10-11
Rat blood	500 µL	7.4
Rabbit blood	500 µL	10
Bone marrow	350 µL	1.7
Buffy coat	500 µL	2-3

IMPORTANT NOTES

- Add the indicated volume of ethanol (96-100%) to **Wash Buffer WB 1** (concentrated) and **Wash Buffer 2** (concentrated) prior to first use:

	#K0761 50 preps	
	Wash Buffer WB 1	**Wash Buffer 2**
Concentrated wash solution	40 mL	23 mL
Ethanol (96-100%)	**4.5 mL**	**39 mL**
Total volume:	44.5 mL	62 mL

After the ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

- Before each RNA purification experiment prepare a fresh aliquot of Lysis Buffer supplemented with β-mercaptoethanol. Add 20 µL of 14.3 M β-mercaptoethanol to each 1 mL of **Lysis Buffer** used. Store at +4°C for up to 1 month.
- Check the **Lysis Buffer** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.
- Wear gloves when handling the **Lysis Buffer and Wash Buffer WB 1** as these solutions contain irritants (*see* p. 8 for SAFETY INFORMATION) and are harmful if they come into contact with skin, or are inhaled or swallowed.
- Unless otherwise indicated all purification steps are preformed at room temperature (15-25°C).
- Centrifugation speed in rpm's is given for 24-place microcentrifuges.
- Typically the purified RNA has an $A_{260/280}$ ratio between 1.9 and 2.1, however, when RNA concentration is lower than 20 ng/µL, deviations from the expected ratio are occasionally observed.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- β-mercaptoethanol
- Pipettes and pipette tips
- Vortex
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Disposable gloves

AVOIDING RIBONUCLEASE CONTAMINATION

RNA purity and integrity is essential for downstream applications. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. Care must be taken not to introduce RNases into the RNA preparation, especially during the column wash and RNA elution steps. General recommendations to avoid RNase contamination include:

- As skin is a common source of RNases, wear gloves when handling reagents and RNA samples. Change gloves frequently.
- Use sterile, disposable RNase-free pipette tips.
- Use reagents designed to remove RNase contamination from nondisposable items (pipettes, centrifuges) and work surfaces.
- Keep all kit components tightly sealed when not in use. After usage, cap bottles immediately.

STARTING MATERIAL HANDLING AND STORAGE

- **Blood sample collection and RNA purification from blood cells should be carried out in the same day**. Samples can be stored at 4°C for no longer than 5 hours. Do not freeze blood samples.
- If it is not possible to process samples the same day, RNA can be preserved in the supplied Lysis Buffer:
  - Centrifuge blood for 5 min 400 × g (~2,000 rpm) at 4°C
  - Discard the supernatant
  - Resuspend the pellet in 600 µL of Lysis Buffer, **mix well**.

Stabilized sample can be stored for 24 h at 4°C, or up to one week at -20°C.

PROTOCOLS

Protocols for RNA purification from buffy coat and bone marrow are described on p.6.

A. Mammalian Whole Blood RNA Purification Protocol

Step	Procedure
1	Centrifuge 50-500 µL of blood for 5 min, 400 × g (~2,000 rpm) at 4°C. Discard the supernatant.
2	Resuspend the pellet in 600 µL of Lysis Buffer, **mix well** by vortexing.
3	Add 450 µL of ethanol (96-100%) and mix by pipetting or vortexing.
4	Transfer half of the prepared lysate to a column inserted in a collection tube. Centrifuge the column for 1 min at 12,000 × g (~11,000 rpm). Discard the flow-through solution and reassemble the column and collection tube. Transfer remaining lysate into the column and centrifuge as before. Discard the collection tube containing the flow-through solution. Place the column into a new 2 mL collection tube (included). Note. Close the bag with GeneJET RNA Purification Columns tightly after each use!
5	Add 700 µL of Wash Buffer WB 1 (with ethanol added). Centrifuge for 1 min at 12,000 × g (~11,000 rpm). Discard the flow-through and place the purification column back into the collection tube.
6	Add 500 µL of Wash Buffer 2 (with ethanol added) to the purification column. Centrifuge for 1 min at 12,000 × g (~11,000 rpm).
7	Add 500 µL of Wash Buffer 2 (with ethanol added) to the purification column. Centrifuge for 2 min at 12,000 × g (~11,000 rpm). *Recommended*: Empty the collection tube. Place the purification column back into the tube and re-spin the column for 1 min. at maximum speed (≥20,000 × g, ≥14,000 rpm). Discard the collection tube containing the flow-through solution and transfer the purification column to an RNase-free 1.5 mL microcentrifuge tube.
8	Add 50 µL of nuclease-free water to the centre of the purification column membrane and centrifuge for 1 min at 12,000 × g (~11,000 rpm).
9	Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20°C until use. Keep the RNA on ice after extraction and while working with it. **Note.** For prolonged storage (more than 1 month) storage at -70°C is recommended.

B. RNA Purification from Bone Marrow

Note. Bone marrow that has been previously frozen does not sediment during centrifugation. Use up to 350 µL of thawed bone marrow and proceed directly to step 2 of the standard mammalian Whole Blood RNA Purification Protocol.

Step	Procedure
1	Take 50-500 µL of **fresh** bone marrow.
2	Centrifuge 50-500 µL of bone marrow for 5 min 400 × g (~2,000 rpm) at 4°C. Discard the supernatant.
3	Proceed to step 2 of the mammalian Whole Blood RNA Purification Protocol on p. 5.

C. RNA Purification from Buffy Coat

Buffy coat is a leukocyte-enriched fraction of whole blood and containes approximately 5-10 times more nucleic acids than an equivalent volume of whole blood. Prepare the buffy coat by centrifuging whole blood at 2,000 × g for 10 minutes at room temperature. After centrifugation, 3 different fractions are distinguishable: the upper clear layer is plasma; the intermediate layer is buffy coat (also called the WBCs), containing concentrated leukocytes; and the bottom layer contains concentrated erythrocytes.

Step	Procedure
1	Centrifuge 10 mL of whole blood at 2,000 × g for 10 minutes at room temperature. Three layers should be visible.
2	Remove upper clear layer by aspiration.
3	Collect approximately **500** µL of the intermediate layer using an automatic pipette, being careful not to disturb the WBCs. Put the WBCs into a fresh tube.
4	Add 10 mL of red blood cell Lysis Solution (10 mM Tris-HCl, pH 7.0, 5 mM $MgCl_2$, 10 mM NaCl) and resuspend WBCs.
5	Centrifuge at 2,000 × g for 10 minutes at room temperature. Remove supernatant. **Do not discard the pellet**.
4	Proceed to step 2 of the mammalian Blood RNA Purification Protocol on p. 5.

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified RNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more blood than indicated in lysis protocols. **Ethanol was not added to the lysate.** Ensure that the ethanol was added to the lysate before applying the sample to the Purification Column. **Ethanol was not mixed with the lysate.** After the addition of ethanol to the lysate mix the sample briefly by vortexing or pipetting. **Ethanol was not added to Wash Buffers.** Ensure that ethanol was added to Wash Buffer WB 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on p.3.
Purified RNA is degraded	**RNase contamination.** To avoid RNase contamination wear gloves during the procedure and change gloves frequently. Use sterile, disposable RNase-free pipette tips. Use reagents designed to remove RNase contamination from nondisposable items (pipettes, centrifuges) and work surfaces. **Inappropriate sample storage conditions.** Blood cells stabilized in Lysis Buffer can be stored at 4°C for no longer than 24 hours or at -20°C for no longer than 7 days. **Purified RNA was not stored properly.** Purified RNA should be used immediately in downstream applications or stored at -20°C for later use. For prolonged storage (more than 1 month) storage at -70°C is recommended. **Lysis buffer does not contain β-mercaptoethanol .** Ensure that β-mercaptoethanol has been added to the lysis buffer.
Inhibition of downstream enzymatic reactions	**Purified RNA contains residual ethanol.** If residual solution is observed in the purification column after treating the column with Wash Buffer 2, empty the collection tube and re-spin the column for an additional 1 min. at maximum speed (≥20,000 × g, ≥14,000 rpm). **Purified RNA contains residual salt.** Use the correct order for the Wash Buffers steps. Always wash the purification column with Wash Buffer WB 1 first and then proceed with Wash Buffer 2.
Column clogging	**Excess starting material was used for lysate preparation.** Reduce the amount of starting material. Do not use more blood or cells than indicated in lysis protocols. **Starting material was not completely lysed.** Reduce the amount of starting material in subsequent preparations.

SAFETY INFORMATION

Lysis Solution

Xn Harmful

Hazard-determining component of labelling: **Guanidinium thiocyanate.**

Risk phrases

R22 Harmful if swallowed.
R36/38 Irritating to eyes and skin.

Safety phrases

S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37 Wear suitable protective clothing and gloves.
S60 This material and its container must be disposed of as hazardous waste.

Wash Buffer WB 1

Xn Harmful

Hazard-determining component of labelling: **Guanidinium hydrochloride.**

Risk phrases

R22 Harmful if swallowed.
R36/38 Irritating to eyes and skin.

Safety phrases

S23 Do not breathe gas/fumes/vapour/spray.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37 Wear suitable protective clothing and gloves.
S60 This material and its container must be disposed of as hazardous waste

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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CERTIFICATE OF ANALYSIS

K0761 GeneJET TM Whole Blood RNA Purification Mini Kit

Packaging Lot: **2667446**
Expiry Date: 31.12.2025 (DD.MM.YYYY)
Storage: at 5±3°C
Note: IMPORTANT Check Individual Components for Storage Conditions

Filling lots for components in package:

Lot	Quantity	Description
2640976	40 mL	Lysis Buffer
2636934	23 mL	Wash Buffer II (concentrated)
01266336	40 mL	Wash Buffer I (concentrated)
2666447	30 mL	Water, nuclease-free
01202691	1 pack	Collection Tubes 2 ml
2631527	1 each	GeneJet RNA purification Columns & Collection Tubes
2632270	1 pack	Collection Tubes 1.5 ml

QUALITY CONTROL

Parameter	Method	Requirement	Result
pH (Relevant kit components)	Measured using a pH meter.	Within range of predetermined specifications	Conforms
Density (Relevant kit components)	Measured using a densitometer.	Within range of predetermined specifications	Conforms
Refractive Index (Relevant kit components)	Measured using a refractometer.	Within range of predetermined specifications	Conforms
Conductivity (Relevant kit components)	Measured using a conductometer.	Within range of predetermined specifications	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė

Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific RevertAid First Strand cDNA Synthesis Kit

#K1622 **100 rxns**

Lot __ **Expiry Date __**

Store at -20°C

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www.thermoscientific.com/onebio

COMPONENTS OF THE KIT

RevertAid First Strand cDNA Synthesis Kit	#K1621 20 rxns	#K1622 100 rxns
RevertAid RT (200 U/µL)	25 µL	120 µL
RiboLock RNase Inhibitor (20 U/µL)	25 µL	120 µL
5X Reaction Buffer 250 mM Tris-HCl (pH 8.3), 250 mM KCl, 20 mM $MgCl_2$, 50 mM DTT	150 µL	500 µL
10 mM dNTP Mix	50 µL	250 µL
Oligo$(dT)_{18}$ Primer, 100 µM	25 µL	120 µL
Random Hexamer Primer, 100 µM,	25 µL	120 µL
Forward GAPDH Primer, 10 µM	20 µL	20 µL
Reverse GAPDH Primer, 10 µM	20 µL	20 µL
Control GAPDH RNA, 0.05 µg/µL	20 µL	20 µL
Water, nuclease-free	2 × 1.25 mL	2 × 1.25 mL

CERTIFICATE OF ANALYSIS

RT-PCR using 100 fg of control GAPDH RNA and control primers generated a prominent 496 bp product on 1% agarose gel after ethidium bromide staining.

Quality authorized by: Jurgita Zilinskiene

Rev.11

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DESCRIPTION

The Thermo Scientific™ RevertAid™ First Strand cDNA Synthesis Kit is a complete system for efficient synthesis of first strand cDNA from mRNA or total RNA templates. The kit uses RevertAid Reverse Transcriptase (RT), which has lower RNase H activity compared to AMV reverse transcriptase. The enzyme maintains activity at 42-50°C and is suitable for synthesis of cDNA up to 13 kb.

The recombinant Thermo Scientific™ RiboLock™ RNase Inhibitor, supplied with the kit, effectively protects RNA from degradation at temperatures up to 55°C.

First strand cDNA synthesized with this system can be directly used as a template in PCR or real-time PCR. It is also ideal for second strand cDNA synthesis or linear RNA amplification. Radioactively and non-radioactively labeled nucleotides can be incorporated into first strand cDNA for use as a probe in hybridization experiments, including microarrays.

STORAGE

All components of the kit should be stored at -20°C. Keep control RNA at -70°C for longer storage.

IMPORTANT NOTES

Avoiding ribonuclease contamination

RNA purity and integrity are essential for synthesis of full-length cDNA. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment.

General recommendations to avoid RNase contamination:

- DEPC-treat all tubes and pipette tips to be used in cDNA synthesis or use certified nuclease-free labware.
- Wear gloves when handling RNA and all reagents, as skin is a common source of RNases. Change gloves frequently.
- Use RNase-free reagents, including high quality water (e.g., Water, nuclease-free, #R0581).
- Use RiboLock™ RNase Inhibitor (provided with the kit) to protect RNA from the activity of RNases.
- Keep all kit components tightly sealed when not in use. Keep all tubes tightly closed during the reverse transcription reaction.

Template RNA

Total cellular RNA isolated by standard methods is suitable for use with the kit. Purified RNA must be free of salts, metal ions, ethanol and phenol to avoid inhibiting the cDNA synthesis reaction. Trace contaminants can be removed by ethanol precipitation of the RNA followed by two washes of the pellet with cold 75% ethanol.

For RT-PCR applications, template RNA must be free of DNA contamination. Prior to cDNA synthesis, RNA can be treated with DNase I, RNase-free (#EN0521) to remove trace amounts of DNA. Always perform a control (RT-minus) reaction which includes all components for RT-PCR except for the reverse transcriptase enzyme.

Removal of genomic DNA from RNA preparations

1. Add to an RNase-free tube:

RNA	1 µg
10X Reaction Buffer with $MgCl_2$	1 µL
DNase I, RNase-free (#EN0521)*	1 µL (1 U)
Water, nuclease-free	to 10 µL

* Do not use more than 1 U of DNase I, RNase-free per 1 µg of RNA.

2. Incubate at 37°C for 30 min.
3. Add 1 µL 50 mM EDTA and incubate at 65°C for 10 min. RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent (1). Alternatively, use phenol/chloroform extraction.
4. Use the prepared RNA as a template for reverse transcriptase.

RNA sample quality

Assess RNA integrity prior to cDNA synthesis. The most common method is denaturing agarose gel electrophoresis followed by ethidium bromide staining. If both 18S and 28S rRNA appear as sharp bands after electrophoresis of total eukaryotic RNA, the RNA is considered to be intact. The 28S rRNA band should be approximately twice as intense as the 18S rRNA. Any smearing of rRNA bands is an indication of degraded mRNA. If this occurs, a new sample of total RNA should be prepared.

RNA quantity

- Use 0.1 ng - 5 µg of total RNA or 1 ng - 500 ng of poly(A) mRNA to generate first strand cDNA as the initial step of a two-step RT-PCR protocol.
- Use 1 µg of isolated mRNA to generate first strand cDNA for second-strand synthesis and subsequent cloning reactions.

PROTOCOLS

I. First Strand cDNA Synthesis

After thawing, mix and briefly centrifuge the components of the kit. Store on ice.

1. Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:

Template RNA	total RNA *or* poly(A) mRNA *or* specific RNA	0.1 ng - 5 µg 10 pg - 0.5 µg 0.01 pg - 0.5 µg
Primer	Oligo $(dT)_{18}$ primer *or* Random Hexamer primer *or* gene-specific primer	1 µL 1 µL 15-20 pmol
Water, nuclease-free		to 12 µL
	Total volume	12 µL

2. *Optional.* If the RNA template is GC-rich or contains secondary structures, mix gently, centrifuge briefly and incubate at 65°C for 5 min. Chill on ice, spin down and place the vial back on ice.
3. Add the following components in the indicated order:

5X Reaction Buffer	4 µL
RiboLock RNase Inhibitor (20 U/µL)	1 µL
10 mM dNTP Mix	2 µL
RevertAid M-MuLV RT (200 U/µL)	1 µL
Total volume	20 µL

4. Mix gently and centrifuge briefly.
5. For oligo$(dT)_{18}$ or gene-specific primed cDNA synthesis, incubate for 60 min at 42°C.
   For random hexamer primed synthesis, incubate for 5 min at 25°C followed by 60 min at 42°C.
   Note. For GC-rich RNA templates the reaction temperature can be increased up to 45°C.
6. Terminate the reaction by heating at 70°C for 5 min.

The reverse transcription reaction product can be directly used in PCR applications or stored at -20°C for less than one week. For longer storage, -70°C is recommended

II. PCR Amplification of First Strand cDNA

The product of the first strand cDNA synthesis can be used directly in PCR or qPCR. The volume of first strand cDNA synthesis reaction mixture should not comprise more than 1/10 of the total PCR reaction volume. Normally, 2 µL of the first strand cDNA synthesis reaction mixture is used as template for subsequent PCR in 50 µL total volume.

(Continued on reverse page)

CONTROL REACTIONS

Positive and negative control reactions should be used to verify the results of the first strand cDNA synthesis steps.

- **Reverse transcriptase minus (RT-) negative control** is important in RT-PCR or RT-qPCR reactions to assess for genomic DNA contamination of the RNA sample. The control RT- reaction contains every reagent for the reverse transcription reaction except for the RT enzyme.
- **No template negative control (NTC)** is important to assess for reagent contamination. The NTC reaction contains every reagent for the reverse transcription reaction except for RNA template.
- **Positive control** RNA template and gene-specific primers are supplied with the kit. The human GAPDH control RNA (1.3 kb) was produced by *in vitro* transcription. The GAPDH-specific control PCR primers are designed to be complementary to human, mouse and rat GAPDH genes and generate 496 bp RT-PCR product. The protocol for the positive control RT-PCR is provided below.

I. Control first strand cDNA synthesis reaction

Mix and briefly centrifuge all components after thawing, keep on ice.

1. Add the following reagents into a sterile, nuclease-free tube on ice in the indicated order:

Control GAPDH RNA (50 ng/µL)	2 µL
Oligo $(dT)_{18}$ Primer *or* Random Hexamer Primer *or* Reverse GAPDH Primer	1 µL
5X Reaction Buffer	4 µL
RiboLock RNase Inhibitor (20 U/µL)	1 µL
10 mM dNTP Mix	2 µL
RevertAid RT (200 U/µL)	1 µL
Water, nuclease-free	9 µL
Total volume	20 µL

2. Mix gently and centrifuge.
3. For oligo$(dT)_{18}$ or gene-specific primed cDNA synthesis, incubate for 60 min at 42°C.
   For random hexamer primed synthesis, incubate for 5 min at 25°C followed by 60 min at 42°C.
4. Terminate the reaction by heating at 70°C for 5 min.
5. Briefly centrifuge and proceed with control PCR amplification.

II. Control PCR amplification

1. Dilute the cDNA generated with the control first strand cDNA reaction 1:1000 in Water, nuclease-free.
2. Gently vortex and briefly centrifuge all PCR reagents after thawing.
3. Place a thin-walled PCR tube on ice and add the following reagents:

cDNA from control RT reaction (1:1000 dilution)	2 µL
10X PCR buffer	5 µL
10 mM dNTP Mix	1 µL (0.2 mM each)
25 mM $MgCl_2$	3 µL
Forward GAPDH Primer	1.5 µL
Reverse GAPDH Primer	1.5 µL
Taq DNA polymerase (5 U/µL)	0.5 µL
Water, nuclease-free	35.5 µL
Total volume	50 µL

4. Perform PCR in a thermal cycler with a heated lid or overlay with 25 µL of mineral oil.

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	94	3 min	1
Denaturation	94	30 s	35
Annealing	58	30 s	
Extension	72	45 s	

5. Load 5-10 µL of the RT-PCR product on 1% agarose gel. A distinct 496 bp PCR product should be visible after ethidium bromide staining.

Reference

1. Wiame, I., et al., Irreversible heat inactivation of DNaseI without RNA degradation, BioTechniques, 29, 252-256, 2000.

TROUBLESHOOTING

Low yield or no RT-PCR product

Degraded RNA template.
RNA purity and integrity is essential for synthesis of full-length cDNA. Always assess the integrity of RNA prior to cDNA synthesis. Sharp 18S and 28S RNA bands should be visible after denaturing agarose gel electrophoresis of total eukaryotic RNA. Follow general recommendations to avoid RNase contamination.

Low template purity.
Trace amounts of agents used in RNA purification protocols may remain in solution and inhibit first strand synthesis, e.g., SDS, EDTA, guanidine salts, phosphate, pyrophosphate, polyamines, spermidine. To remove trace contaminants, re-precipitate the RNA with ethanol and wash the pellet with 75% ethanol.

Insufficient template quantity.
Increase the amount of template to the recommended level. Following DNase I treatment, terminate the reaction by heat inactivation in the presence of EDTA (to bind magnesium ions), RNA hydrolyzes during heating in the absence of a chelating agent (1).

Incorrect primer choice.
Use the correct primer for the RNA template. Use the random hexamer primer instead of the oligo$(dT)_{18}$ primer with bacterial RNA or RNA without a poly(A) tail. Ensure sequence-specific primers are complementary to 3'-end of the template RNA.

GC rich template.
If the RNA template is GC rich or is known to contain secondary structures, increase the temperature of the reverse transcription reaction up to 45°C.

RT-PCR product longer than expected

RNA template is contaminated with DNA.
Amplification of genomic DNA containing introns. Perform DNase I digestion prior reverse transcription. To avoid amplification of genomic DNA, design PCR primers on exon-intron boundaries.

RT-PCR product in negative control

RNA template is contaminated with DNA.
PCR product in the negative control (RT-) indicates the reaction is contaminated with DNA. Perform DNase I digestion prior reverse transcription.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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ThermoFisher SCIENTIFIC
The world leader in serving science

CERTIFICATE OF ANALYSIS

K1622 RevertAid 1st cDNA Synth Kit

Packaging Lot: **2698117**
Expiry Date: 31.10.2024 (DD.MM.YYYY)
Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
2677696	0.12 mL	RiboLock RI
2663398	24 kU	RevertAid RT 200u/µl
2687794	0.02 mL	Control GAPDH RNA
2690790	0.02 mL	Forward Control Primer
2690210	0.12 mL	Oligo(dT)18 Primer
2663390	0.12 mL	Random Hexamer Primer
2663471	0.02 mL	Reverse Control Primer
2635416	0.5 mL	5X Reaction Buffer
2675060	2 x 1.25 mL	Water, Nuclease-free
2632584	0.25 mL	dNTP Mix 10 mM

QUALITY CONTROL

Parameter	Method	Requirement	Result
Functional testing	RT-PCR using 100 fg of control GAPDH RNA and control primers generated a prominent 496 bp product on agarose gel.	Conforms	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė

6X TriTrack DNA Loading Dye

Catalog Number R1161
Pub. No. MAN0013168 **Rev.** B.00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

Contents and storage

Contents	Amount	Storage
6X TriTrack DNA Loading Dye	5 x 1 mL	room temperature or at 4 ºC for periods up to 12 months. For longer periods, store at -20 ºC

Description

The 6X Thermo Scientific TriTrack DNA Loading Dye is used to prepare DNA markers and samples for loading on agarose or polyacrylamide gels. The optimized solution contains three dyes: bromophenol blue, xylene cyanol FF and orange G for visual three-color tracking of DNA migration during electrophoresis. The presence of glycerol in the solution ensures that the DNA in the ladder and sample forms a layer at the bottom of the well; the EDTA included in the solution binds divalent metal ions and inhibits metal dependent nucleases.

Composition

10 mM Tris-HCl (pH 7.6)
0.03 % bromophenol blue
0.03 % xylene cyanol FF
0.15 % orange G
60 % glycerol
60 mM EDTA.

Usage Recommendations

1. Add 1 volume of 6X TriTrack™ DNA Loading Dye to 5 volumes of DNA sample.
2. Mix well, spin down and load.

Note

In 1 % agarose gels bromophenol blue co-migrates with ~300 bp DNA, while xylene cyanol FF co-migrates with ~4000 bp DNA. Orange G co-migrates with smallest fragments, i.e. ~50 bp.

Limited product warranty

Life Technologies Corporation and/or it 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**.

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Company code 122351387, VAT code LT223513811

ThermoFisher SCIENTIFIC
The world leader in serving science

CERTIFICATE OF ANALYSIS

R1161 **6X TriTrack DNA Loading Dye**
Packaging Lot: **2687765**
Expiry Date: 31.01.2027 (DD.MM.YYYY)
Storage: at 5±3°C

Filling lots for components in package:

Lot	Quantity	Description
2634130	5 x 1 mL	6X TriTrack DNA Loading Dye

QUALITY CONTROL

Parameter	Method	Requirement	Result
Electrophoretic mobility	Tested for DNA sample preparation prior to agarose gel electrophoresis.	Conforms	Conforms
$A_{595\ nm}$	Spectrophotometrical measurement.	0.598 ± 0.06	Conforms
$A_{600\ nm}$	Spectrophotometrical measurement.	0.609 ± 0.06	Conforms
$A_{605\ nm}$	Spectrophotometrical measurement.	0.604 ± 0.06	Conforms
A_{595}/A_{600}	Spectrophotometrical measurement.	≤ 1	Conforms
A_{605}/A_{600}	Spectrophotometrical measurement.	≤ 1	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė

thermo scientific

PRODUCT INFORMATION

Thermo Scientific GeneRuler 50 bp DNA Ladder, ready-to-use

Pub. No. MAN0013018
Rev. Date 12 February 2018 (Rev. C.00)

Components	#SM0373
GeneRuler 50 bp DNA Ladder, ready-to-use, 0.1 µg/µL	50 µg (for 100 applications)
6X TriTrack DNA Loading Dye	1 mL

Store at room temperature or at 4°C for up to 6 months. For longer periods store at -20°C.

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For Research Use Only. Not for use in diagnostic procedures.

Description

Thermo Scientific™ GeneRuler™ 50bp DNA Ladder, ready-to-use, is designed for sizing and approximate quantification of wide range double-stranded DNA on agarose and polyacrylamide gels. The ladder is composed of thirteen chromatography-purified individual DNA fragments (in base pairs): 1000, 900, 800, 700, 600, **500**, 400, 300, **250**, 200, 150, 100, 50. It contains two reference bands (500 and 250 bp) for easy orientation. The ladder is ready to use – it is premixed with 6X TriTrack DNA Loading Dye for direct loading on gel.

Storage and Loading Buffer

10 mM Tris-HCl (pH 7.6), 10 mM EDTA, 0.005% bromophenol blue, 0.005% xylene cyanol FF, 0.025% orange G and 10% glycerol.

6X TriTrack DNA Loading Dye

10 mM Tris-HCl (pH 7.6), 0.03% bromophenol blue, 0.03% xylene cyanol FF, 0.15% orange G, 60% glycerol and 60 mM EDTA.

Protocol for Loading

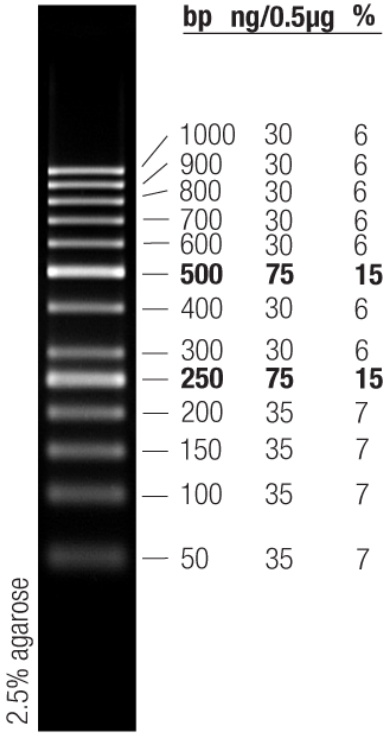
Step 1: Mix gently

Step 2: Load 1 µL per 1 mm gel lane

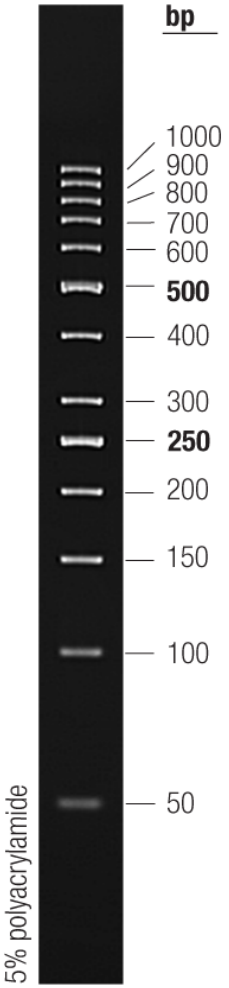
Recommendations

- Do not heat before loading.
- Dilute your DNA sample with the 6X TriTrack DNA Loading Dye (#R1161, supplied with the ladder): mix 1 volume of the dye solution with 5 volumes of the DNA sample;
- Load the same volumes of the DNA sample and the DNA ladder;
- For quantification, adjust the concentration of the sample to equalize it approximately with the amount of DNA in the nearest band of the ladder.
- For DNA band visualization with SYBR™ Green and other intercalating dyes, do not add the dyes into the sample, use gel staining after electrophoresis or include dyes into agarose gel to avoid aberrant DNA migration.
- **Important note:** For DNA bands visualization with GelRed™ use gel staining after electrophoresis to avoid aberrant DNA migration.

GeneRuler 50 bp DNA Ladder, ready-to-use



0.5 µg/lane, 8 cm length gel,
1X TBE, 5 V/cm, 1 h



0.5 µg/lane, 20 cm length gel,
1X TAE, 8 V/cm, 3 h

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PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermofisher.com for Material Safety Data Sheet of the product.

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CERTIFICATE OF ANALYSIS

SM0373 **GeneRuler 50bp DNA Ladder, rtu**
Packaging Lot: **2675818**
Expiry Date: 31.01.2027 (DD.MM.YYYY)
Storage: at -20±5°C

Filling lots for components in package:

Lot	Quantity	Description
2651284	50 µg	GeneRuler 50bp DNA Ladder, rtu
2633915	1 mL	6X TriTrack DNA Loading Dye

QUALITY CONTROL

Parameter	Method	Requirement	Result
Appearance	Visual inspection.	Dark solution	Conforms
Identity	1 µg of the ladder is analyzed by agarose gel electrophoresis in parallel with the same composition ladder from a previous bulk lot.	13 fragments are visible and correspond to those from the previous bulk lot	Conforms
Deoxyribonucleases	Incubation of DNA Ladder at elevated temperature (37 °C) and analysis on agarose gel.	No changes in bands intensity, no smearing is observed after incubation	Conforms

ISO CERTIFICATION
Manufactured by Thermo Fisher Scientific Baltics UAB, in compliance with ISO 9001 and ISO 13485 certified quality management system.

Quality authorized by QC: J. Žilinskienė