USER GUIDE

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PureLink® Genomic DNA Kits

For purification of genomic DNA

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Contents

Experienced Users' Mini Kit Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink® Genomic DNA Mini Kit. For more details, refer to this manual.

Step	Procedure		
Preparing lysates	Prepare the lysate using an appropriate sample preparation protocol as follows:		
	Sample	**Amount**	**Page no.**
	Mammalian cells, tissues and mouse tail	5×10^6 cells, ≤25 mg tissue (≤10 mg spleen), 0.5–1 cm tail	16
	Blood	≤1 mL non-nucleated blood (need additional reagents, see page 17)	17
		5–10 µL nucleated blood	
	Bacteria	$\leq 2 \times 10^9$ cells	18
	Yeast cells	$\leq 5 \times 10^7$ cells	19
	Buccal swab	Human buccal swab	19
	FFPE tissue	1–8 sections of 5–15 µm thick of about 20–50 mm^2 area	20
	Oragene™ preserved saliva	≤1 mL	21
Binding DNA	1. Remove a PureLink® Spin Column in a Collection Tube from the package. 2. **Load** the lysate (~640 µL) with Lysis/Binding Buffer and ethanol prepared as described on pages 16–21 to the PureLink® Spin Column. 3. Centrifuge the column at 10,000 × *g* for 1 minute at room temperature. 4. Discard the collection tube and place the spin column into a new collection tube. 5. Proceed to **Washing DNA**.		
Washing DNA	1. **Wash** the column with 500 µL Wash Buffer 1 prepared with ethanol (page 23). 2. Centrifuge the column at 10,000 × *g* for 1 minute at room temperature. Discard the collection tube and place column into a new collection tube. 3. **Wash** the column with 500 µL Wash Buffer 2 prepared with ethanol (page 23). 4. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard the collection tube. 5. Proceed to **Eluting DNA**.		
Eluting DNA	1. Place the spin column in a sterile 1.5-mL microcentrifuge tube. 2. **Elute** the DNA with 25–200 µL of PureLink® Genomic Elution Buffer. See **Elution Parameters** (page 13) to choose a suitable elution volume for your needs. 3. Incubate the column at room temperature for 1 minute. 4. Centrifuge the column at maximum speed for 1 minute at room temperature. *The tube contains purified DNA.* 5. If desired, perform a second elution to increase recovery which lowers the overall concentration. *The tube contains purified DNA.* Remove and discard the column. 6. Use the purified gDNA for the desired downstream application. Store the purified gDNA at 4ºC for short-term or –20°C for long-term storage.		

Experienced Users' 96 Kit Procedure

Introduction

This quick reference sheet is included for experienced users of the PureLink® 96 Genomic DNA Kit. For more details, refer to this manual.

Step	Procedure		
Preparing lysates	Prepare the lysate using an appropriate sample preparation protocol as follows:		
	Sample	**Amount**	**Page no.**
	Mammalian cells, tissues and mouse tail	5×10^6 cells, ≤25 mg tissue (≤10 mg spleen), 0.5–1 cm tail	27, 28
	Blood	≤200 µL non-nucleated blood	27
		5–10 µL nucleated blood	
	Bacteria	$\leq 2 \times 10^9$ cells	30
	Yeast cells	$\leq 5 \times 10^7$ cells	31
	FFPE tissue	1–8 sections of 5–15 µm thick of about 20–50 mm^2 area	32
	Oragene™ preserved Saliva	≤200 µL	33
	Buccal Swab	Human buccal swabs	33
Purification using centrifugation	1. Assemble the PureLink® gDNA Binding Plate onto a new or used 96 Deep Well Plate. 2. Transfer each lysate (~640 µL) to a well of the PureLink® gDNA Binding Plate using a multichannel pipettor. Cover any unused wells with Foil Tape. 3. Centrifuge the stacked plates at ≥2250 × *g* for 5–10 minutes. 4. Discard flow through and reassemble the Binding Plate onto the 96 Deep Well Plate. 5. Add 500 µL Wash Buffer 1 prepared with ethanol (page 35) into each well of the binding plate. 6. Centrifuge the stacked plates at ≥2250 × *g* for 5–10 minutes. 7. Discard the flow through and reassemble the plate stack. 8. Add 500 µL Wash Buffer 2 prepared with ethanol (page 35) into each well of the binding plate. 9. Centrifuge stacked plates at ≥2250 × *g* for 15 minutes to completely dry the membrane. **Note:** To ensure the complete drying of the membrane, do not seal the plate. 10. Discard flow through and reassemble the binding plate onto a **new** 96 Deep Well Plate. 11. Add 50–200 µL PureLink® Genomic Elution Buffer to the center of the membrane in each well and incubate the plate for 1 minute at room temperature. **Note:** Review **Elution Parameters** (page 13) to choose a suitable elution volume. 12. Centrifuge the stacked plates at ≥2250 × *g* for 3 minutes. *The purified gDNA is eluted in the Deep Well Plate.* 13. If desired, perform a second elution to increase recovery which lowers the overall concentration. 14. Use the purified gDNA for the desired downstream application. To store the purified gDNA, cover the wells with a Foil Tape, and store at 4ºC for short-term or –20°C for long-term storage.		

Experienced Users' 96 Kit Procedure, Continued

Step	Procedure
Purification using vacuum manifold	1. Assemble the vacuum manifold as per the manufacturer's instructions. Brief instructions for assembling the PureLink® Vacuum Manifold with adaptors and PureLink® Genomic Wash Plate to prevent cross contamination are described on page 37. 2. Place the PureLink® gDNA Binding Plate onto the vacuum manifold. 3. Transfer the lysates (~640 µL) from each well of the Deep-Well Plate to a fresh well in the Binding Plate. Cover unused wells with Foil Tape. 4. Apply vacuum at room temperature until the lysate completely passes through the filter plate and release vacuum. 5. Add 1 mL Wash Buffer 1 prepared with ethanol (page 35) into each well of the Binding Plate. 6. Apply vacuum for 2 minutes at room temperature. Release vacuum. 7. Add 1 mL Wash Buffer 2 prepared with ethanol (page 35) into each well of the Binding Plate. 8. Apply vacuum for 2 minutes at room temperature. Release vacuum. 9. Disassemble the manifold to remove and discard the wash plate. Tap the Binding Plate on paper towels to remove any residual Wash Buffer from the nozzles. Reassemble the manifold with the Binding Plate. 10. Apply vacuum for 10 minutes at room temperature to dry the membrane. Release vacuum. 11. Disassemble the manifold to remove the waste tray. Discard the waste tray contents. 12. Assemble the vacuum manifold for elution as per the manufacturer's instructions. See page 38 for brief instructions on assembling various vacuum manifolds with different adaptors and elution plates. 13. Place the PureLink® gDNA Binding Plate onto the vacuum manifold. 14. Add 100–200 µL of PureLink® gDNA Elution Buffer to the center of the membrane and incubate the plate for 1 minute at room temperature. **Note:** Review **Elution Parameters** on page 13 to choose a suitable elution volume. 15. Apply vacuum for 2 minutes at room temperature. Release vacuum. Disassemble the vacuum manifold to remove the elution plate. *The purified gDNA is eluted into the elution plate.* 16. Use the purified gDNA for the desired downstream application. To store the purified gDNA, cover the wells with a Foil Tape, and store at 4ºC for short-term or –20°C for long-term storage.

Kit Contents and Storage

Important

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

Types of Products

This manual is supplied with the following products:

Product	Quantity	Catalog No.
PureLink® Genomic DNA Mini Kit	50 preps	K1820-01
	250 preps	K1820-02
PureLink® 96 Genomic DNA Kit	4 × 96 preps	K1821-04

Shipping and Storage

All components of the PureLink® Genomic DNA Kits are shipped at room temperature.

Upon receipt, store all components at room temperature.

Note: The Proteinase K solution and RNase A are stable for 1 year when stored at room temperature. For long-term storage (>1 year) or if room temperature is >25ºC, store the Proteinase K solution and RNase A at 4ºC.

PureLink® Genomic DNA Mini Kit Contents

The components included in the PureLink® Genomic DNA Mini Kits are listed in the following table.

Component	K1820-01	K1820-02
	50 preps	250 preps
PureLink® Genomic Lysis/Binding Buffer	10 mL	50 mL
PureLink® Genomic Digestion Buffer	9 mL	45 mL
PureLink® Genomic Wash Buffer 1	10 mL	50 mL
PureLink® Genomic Wash Buffer 2	7.5 mL	37.5 mL
PureLink® Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA)	10 mL	50 mL
RNase A (20 mg/mL) in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA	1 mL	5 mL
Proteinase K (20 mg/mL) in storage buffer (proprietary)	1 mL	5 mL
PureLink® Spin Columns with Collection Tubes	50 each	5 × 50 each
PureLink® Collection Tubes (2.0 mL)	100	5 × 100

PureLink® 96 Genomic DNA Kit Contents

The components included in the PureLink® 96 Genomic DNA Kit are listed in the following table.

Note: Some reagents in the kit may be provided in excess of the amount needed.

Component	Quantity
PureLink® Genomic Lysis/Binding Buffer	80 mL
PureLink® Genomic Digestion Buffer	70 mL
PureLink® Genomic Wash Buffer 1	2 × 100 mL
PureLink® Genomic Wash Buffer 2	2 × 75 mL
PureLink® Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA)	160 mL
RNase A (20 mg/mL) in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA	8 mL
Proteinase K (20 mg/mL) in storage buffer (proprietary)	8 mL
PureLink® Genomic DNA Binding Plate	4 plates
PureLink® Genomic DNA Wash Plate	4 plates
96 Deep Well Plate	2 × 6 plates
Foil Tape	20/pack

Introduction

Overview

Introduction

The PureLink® Genomic DNA Kits allow rapid and efficient purification of genomic DNA. The kit is designed to efficiently isolate genomic DNA from mammalian cells and tissues, mouse/rat tail, blood samples, buccal swabs, bacteria, yeast, FFPE (formalin-fixed paraffin-embedded) tissue, and Oragene™ preserved saliva. After preparing the lysates, the DNA is rapidly purified from lysates using a spin column based centrifugation procedure or high throughput isolation using 96-well plates with a vacuum manifold or automated liquid handling workstations.

The isolated DNA is 20–50 kb in size and is suitable for PCR, restriction enzyme digestion, and Southern blotting.

System Overview

The PureLink® Genomic DNA Kits are based on the selective binding of DNA to silica-based membrane in the presence of chaotropic salts.

The lysate is prepared from a variety of starting materials such as tissues, cells, or blood. The cells or tissues are digested with Proteinase K at 55°C using an optimized digestion buffer formulation that aids in protein denaturation and enhances Proteinase K activity. Any residual RNA is removed by digestion with RNase A prior to binding samples to the silica membrane.

The lysate is mixed with ethanol and PureLink® Genomic Binding Buffer that allows high DNA binding PureLink® Spin Column (Mini Kit) or Binding Plate (96 Kit). The DNA binds to the silica-based membrane in the column or plate and impurities are removed by thorough washing with Wash Buffers. The genomic DNA is then eluted in low salt Elution Buffer.

Advantages

The advantages of using PureLink® Genomic DNA Kits are:

- Rapid and efficient purification of genomic DNA from a variety of samples such as mammalian cells and tissue, blood samples, mouse tails, buccal swabs, bacteria, yeast, FFPE tissue, and Oragene™ preserved saliva
- Designed to rapidly purify high-quality DNA using spin column or 96-well plate format
- Automation using standard robotic systems (96 kit) with no sample cross contamination
- Simple lysis of cells and tissues with Proteinase K without the need for any mechanical lysis
- Minimal contamination from RNA
- Reliable performance of the purified DNA in PCR, restriction enzyme digestion, and Southern blotting

Mini Kit Specifications

Starting Material:	Varies (see page 12)
Binding Capacity:	~0.5 mg nucleic acid
Column Reservoir Capacity:	800 µL
Collection Tube Capacity:	2.0 mL (~700 µL without contacting column)
Centrifuge Compatibility:	Capable of centrifuging >10,000 × *g*
Elution Volume:	25–200 µL
DNA Yield:	Varies (see page 40)
DNA Size:	20–50 kb

96 Kit Specifications

Dimensions:	Standard SBS (Society for Biomolecules Screening) footprint
Starting Material:	Varies (see page 12)
Binding Capacity:	~0.5 mg nucleic acid
Binding Plate Capacity:	1 mL
Deep-Well Plate Capacity:	1.0 mL (0.75 mL without contacting nozzles)
Centrifuge Compatibility:	Capable of centrifuging at ≥2,250 × *g* Bucket depth 5.75 cm
Elution Volume:	50–200 µL
DNA Yield:	Varies
DNA Size:	20–50 kb

Methods

General Guidelines

Introduction

General guidelines for using the PureLink® Genomic DNA Kits are described in the following sections. Review this section before starting the purification procedure.

Choose the appropriate purification protocol based on the type of kit you have purchased:

Kit	Page no.
PureLink® Genomic DNA Mini Kits (K1820-01, K1820-02)	14
PureLink® 96 Genomic DNA Kit (K1821-04)	25

To obtain high-quality genomic DNA, follow the guidelines recommended on page 11.

Follow the listed recommendations to obtain the best results:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
- Ensure that no DNases are introduced into the sterile solutions of the kit
- Make sure all equipment that comes in contact with DNA is sterile including pipette tips and microcentrifuge tubes
- Do not vortex the samples for more than 5–10 seconds at each vortexing step to avoid extensive shearing of DNA
- To minimize DNA degradation, perform lysate preparation steps quickly, and avoid repeated freezing and thawing of DNA samples

RNase A Digestion

RNase A digestion is performed during sample preparation to degrade RNA present in the sample and minimize RNA contamination in the purified DNA sample. RNA contamination also inflates the DNA content measured at 260 nm.

RNase A is supplied with the kit and an RNase digestion step is included during sample preparation protocols.

If RNA content of the sample is minimal (e.g., mouse tail) and RNA contamination *does not* interfere with any downstream applications of the purified DNA, you may omit the RNase digestion step during sample preparation.

Proteinase K Digestion

The Proteinase K is used for efficient lysis of tissues/cells. Proteinase K digestion is performed using an optimized buffer formulation, PureLink® Genomic Digestion Buffer, for optimal enzymatic activity.

Sample Amount

There are different protocols for preparing lysates depending on the starting material (sample). Based on your sample, choose an appropriate lysate preparation protocol from the following table.

The PureLink® Genomic DNA Kits are suitable for isolating genomic DNA from a variety of samples using the recommended sample amount (see table below).

Note: If you start with less amount of sample, the yield of DNA may also be lower.

To obtain high yield of DNA and minimize DNA degradation, collect the sample and proceed immediately to sample preparation or freeze the sample in liquid nitrogen immediately after collection.

Sample	Amount
Mammalian cells	5×10^6 cells (suspension or adherent cells)
Mammalian tissues	≤25 mg (≤10 mg for spleen)
Mouse or rat tail	1 cm (mouse); 0.5 cm (rat)
Buccal swab	Human buccal swab
Nonnucleated whole blood (e.g., human, mouse)	≤200 µL (single pass) ≤1 mL (multiple pass, Mini Kit only)
Nucleated whole blood (e.g., bird)	5–10 µL
Blood spot on paper	2–5 punches (2–3 mm in size)
Gram negative bacteria (e.g., *E. coli*)	$\leq 2 \times 10^9$ cells
Gram positive bacteria (e.g., Bacillus)	$\leq 2 \times 10^9$ cells
Yeast cells	$\leq 5 \times 10^7$ cells
FFPE tissue	1–8 sections of 5–15 µm thick with a tissue surface area of 20–50 mm^2.
Oragene™ preserved saliva	≤4 mL (Mini Kit); ≤200 µL (96 Kit)

Whole Blood Sample

The PureLink® Genomic DNA Kits are designed to purify genomic DNA from the following whole blood samples:

- Fresh or frozen whole blood
- Fresh or frozen whole blood collected in the presence of anti-coagulants such as EDTA or citrate
- Dried blood spots on paper such as FTA® card (Whatman) or S&S 903.

Elution Parameters

Elution Buffer

The genomic DNA is eluted using PureLink® Genomic Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA). Alternatively, Tris Buffer (10 mM Tris-HCl, pH 8.0–9.0) or sterile water can be used, if EDTA inhibits downstream reactions.

Elution Buffer Volume

The genomic DNA is eluted in 25–200 µL (Mini Kit) or 50–200 µL (96 Kit) of PureLink® Genomic Elution Buffer. You can change the volume of elution buffer to obtain genomic DNA in the desired final concentration. Use the graph shown below to determine the most appropriate elution conditions for your application. For increased DNA yield, use a higher volume of elution buffer. For increased DNA concentration, use a lower volume of elution buffer.

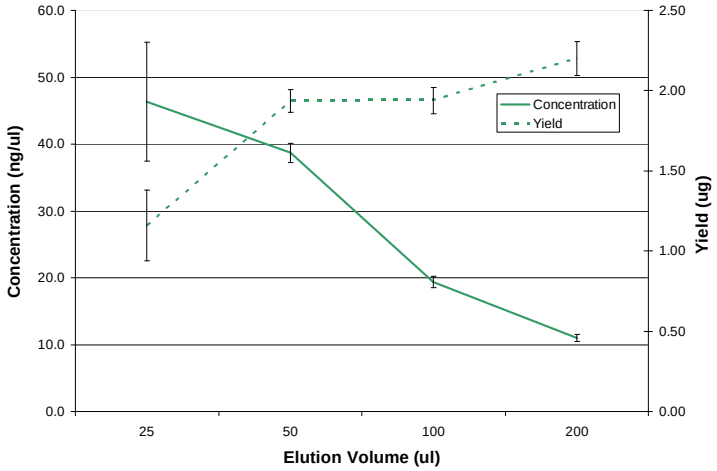


Figure Legend: Genomic DNA was purified from 100 µL blood samples with the PureLink® Genomic DNA Mini Kit using different elution volumes.

Number of Elutions

Using 50 µL and 100 µL elution buffer volume, the first elution recovers ~80% and 90% of bound genomic DNA, respectively. To maximize genomic DNA recovery, you may perform a second elution to recover the remaining 10–15% gDNA. To prevent dilution of the gDNA sample perform the second elution using the same volume of buffer used for first elution. Avoid contact of the spin column with the eluate by using different tubes for the two-elution steps.

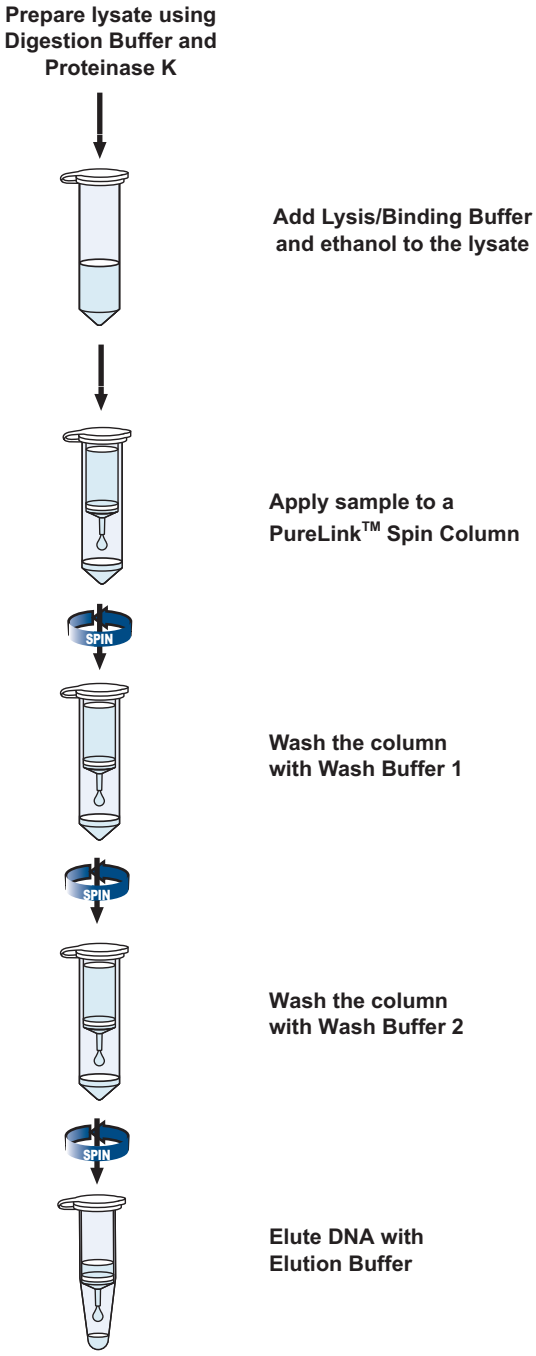
Note: Sufficient PureLink® Genomic Elution Buffer is included to perform up to 2 × 100 µL elution per sample. If you wish to perform >2 × 100 µL elution per sample, you need to purchase additional PureLink® Genomic Elution Buffer (page 43) available separately.

Purification Procedure Using Mini Kit

Experimental Overview

Introduction

The procedure for purifying genomic DNA using the PureLink® Genomic DNA Mini Kits is illustrated in the following flow chart.



Preparing Lysates–Mini Kit

Introduction

Instructions for preparing lysates from mammalian cells and tissues, mouse tail, buccal swabs, blood, bacteria, yeast, FFPE tissues, and Oragene™ preserved saliva are described in the following sections.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 11.

Important

Make sure there is no precipitate visible in PureLink® Genomic Digestion Buffer or PureLink® Genomic Lysis/Binding Buffer. If any precipitate is visible in the buffers, warm the buffers at 37°C for 3–5 minutes and mix well to dissolve the precipitate before use.

Materials Needed

Components supplied by the user

- 96–100% ethanol
- Sample for DNA isolation (see page 12 for recommended starting amount)
- Phosphate Buffered Saline (PBS) for mammalian cell lysate (page 43)
- Lysozyme and Lysozyme Digestion Buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) for bacterial cell lysate
- Zymolase Buffer (1 M sorbitol, 10 mM sodium EDTA, 14 mM β-mercaptoethanol) and Zymolase (lyticase) enzyme for yeast lysate
- CitriSolv™ Clearing Agent (Fisher cat. no. 22-143-975) or xylene for FFPE tissue
- 3 M sodium acetate (pH 5–5.5) and 2.8 mL isopropanol for Oragene™ samples
- Sterile, DNase–free microcentrifuge tubes
- Water baths or heat blocks

Components supplied with the Kit

- PureLink® Genomic Lysis/Binding Buffer
- PureLink® Genomic Digestion Buffer
- Proteinase K (20 mg/mL)
- RNase A (20 mg/mL)

Preparing Lysates–Mini Kit, Continued

Mammalian Cells Lysate

Use the following protocol to prepare lysate from mammalian cells.

1. Set a water bath or heat block at 55°C.
2. For adherent cells (≤5 × 10^6 cells), remove the growth medium from the culture plate and harvest cells by trypisinization or a method of choice.

   For suspension cells (≤5 × 10^6 cells), harvest cells and centrifuge the cells at 250 × *g* for 5 minutes to pellet cells. Remove the growth medium.
3. Resuspend the cells from Step 2 in 200 µL PBS.
4. Add 20 µL Proteinase K (supplied with the kit) to the sample.
5. Add 20 µL RNase A (supplied with the kit) to the sample, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
6. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
7. Incubate at 55°C for 10 minutes to promote protein digestion.
8. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
9. Proceed immediately to **Binding DNA** (page 23).

Mammalian Tissue and Mouse/Rat Tail Lysate

Use the following protocol to prepare lysate from mammalian tissues and mouse/rat tails.

1. Set a water bath or heat block at 55°C.
2. Place the following amount of mammalian tissue or tail into a sterile microcentrifuge tube:
   - ≤25 mg of minced mammalian tissue
   - ≤10 mg minced spleen tissue
   - 1 cm mouse or 0.5 cm rat tail clip
3. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit) to the tube. Ensure the tissue is completely immersed in the buffer mix.

   Note: When processing multiple samples, prepare a master Digestion Buffer Mix by mixing 180 µL Digestion Buffer and 20 µL Proteinase K for each sample.
4. Incubate at 55°C with occasional vortexing until lysis is complete (1–4 hours). For mouse tails or larger tissue pieces, you may perform overnight digestion.
5. To remove any particulate materials, centrifuge the lysate at maximum speed for 3 minutes at room temperature. Transfer supernatant to a new, sterile microcentrifuge tube.
6. Add 20 µL RNase A (supplied in the kit) to the lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
7. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by vortexing.
8. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds.

   Note: When processing multiple samples, prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol per sample.
9. Proceed immediately to **Binding DNA** (page 23).

Blood Lysate

Use the following protocol to prepare lysate from blood samples (nucleated or nonnucleated).

Note: If you are processing >200 µL blood sample, you need to purchase additional PureLink® Genomic Lysis/Binding Buffer and Proteinase K (page 43).

1. Set a water bath or heat block at 55°C.
2. To a sterile microcentrifuge tube, add ≤200 µL fresh or frozen blood sample (if using <200 µL blood sample, adjust the sample volume to 200 µL using PBS).

   For processing blood samples >200 µL and ≤1 mL, scale up all reagent volumes accordingly.
3. Add 20 µL Proteinase K (supplied with the kit) to the sample.
4. Add 20 µL RNase A (supplied with the kit) to the sample, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
5. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
6. Incubate at 55°C for 10 minutes to promote protein digestion.
7. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
8. Proceed immediately to **Binding DNA** (page 23).

Blood Spots

Use the following protocol to prepare lysate from dried blood spots.

1. Set a water bath or heat block at 55°C.
2. Place 2–5 punches of dried blood spot (2–3 mm in size) in a sterile microcentrifuge tube.
3. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit) to the tube. Mix well by vortexing. Ensure the pieces are completely immersed in buffer.
4. Incubate at 55°C with occasional vortexing for 30 minutes.
5. Centrifuge the sample at maximum speed for 2–3 minutes at room temperature to pellet paper fibers. Transfer the sample to a clean, sterile microcentrifuge tube.
6. Add 20 µL RNase A (supplied in the kit) to the lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
7. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
8. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
9. Proceed immediately to **Binding DNA** (page 23).

Gram Negative Bacterial Cell Lysate

Use the following protocol to prepare Gram negative bacterial cell lysate.

1. Set a water bath or heat block at 55°C.
2. Harvest up to 2×10^9 Gram negative (~1 mL of overnight *E. coli* culture) by centrifugation. If you are using a frozen cell pellet, proceed to Step 3.
3. Resuspend the cell pellet in 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K (supplied with the kit) to lyse the cells. Mix well by brief vortexing.
4. Incubate the tube at 55°C with occasional vortexing until lysis is complete (30 minutes to up to 4 hours).
5. Add 20 µL RNase A (supplied with the kit) to the lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
6. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by vortexing to obtain a homogenous solution.
7. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
8. Proceed to **Binding DNA** (page 23).

Gram Positive Bacterial Cell Lysate

Use the following protocol to prepare Gram positive bacterial cell lysate.

1. Set two water baths or heat blocks at 37ºC and 55°C, respectively.
2. Prepare Lysozyme Digestion Buffer (see recipe on page 15). To ~200 µL Lysozyme Digestion Buffer/sample, add **fresh** Lysozyme to obtain a final Lysozyme concentration of 20 mg/mL.
3. Harvest up to 2×10^9 Gram positive cells by centrifugation. If you are using a frozen cell pellet, proceed to Step 3.
4. Resuspend the cell pellet in 180 µL Lysozyme Digestion Buffer containing Lysozyme from Step 2. Mix well by brief vortexing.
5. Incubate at 37ºC for 30 minutes.
6. Add 20 µL Proteinase K (supplied with the kit). Mix well by brief vortexing.
7. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by brief vortexing.
8. Incubate at 55°C for 30 minutes.
9. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
10. Proceed to **Binding DNA** (page 23).

Yeast Cells Lysate

Use the following protocol to prepare lysate from yeast cells.

1. Set 2 water baths or heat blocks at 37°C and 55°C, respectively.
2. Prepare **fresh** Zymolase Buffer (see page 15). You need 500 µL buffer per sample.
3. Harvest up to 5×10^7 yeast cells by centrifugation. If you are using a frozen cell pellet, proceed to Step 4.
4. Resuspend the cell pellet in 500 µL Zymolase Buffer. Add 15 units Zymolase (lyticase) enzyme and incubate at 37°C for 1 hour to generate spheroplasts.
5. Centrifuge at 3000 × *g* for 10 minutes at room temperature to pellet the spheroplasts. Discard the supernatant.
6. Resuspend the spheroplasts in 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K (supplied with the kit). Mix well by brief vortexing.
7. Incubate at 55°C for 45 minutes.
8. Add 20 µL RNase A (supplied in the kit) to the lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
9. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by brief vortexing to obtain a homogenous solution.
10. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.

    Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
11. Proceed immediately to **Binding DNA** (page 23).

Human Buccal Swab Lysate

Use the following protocol to prepare lysate from human buccal cell swabs.

1. Set a water bath or heat block at 55°C.
2. Place the buccal swab in a sterile, 2-mL microcentrifuge tube. Add 400 µL (for cotton and Dacron swab) or 600 µL (for Omni Swab) PBS to the sample.
3. Add 20 µL Proteinase K into a sterile microcentrifuge tube capable of holding three times the volume of lysate (for example, if you plan to process 600 µL lysate, use a microcentrifuge tube capable of holding 1800 µL).
4. Transfer 200–600 µL swab lysate to the microcentrifuge tube containing Proteinase K (Step 3). Mix well by pipetting.
5. Add an equal volume of PureLink® Genomic Lysis/Binding Buffer to the lysate and mix well by brief vortexing.

   For example, if you are processing 200 µL lysate, add 200 µL PureLink® Genomic Lysis/Binding Buffer.
6. Incubate at 55°C for at least 10 minutes.
7. Centrifuge briefly to collect any lysate from the tube caps.
8. Add 200 µL 96–100% ethanol to the tube. Mix well by vortexing for 5 seconds to yield a homogenous solution.
9. Proceed immediately to **Binding DNA** (page 23).

FFPE Tissue Lysate

Prepare lysate from FFPE (formalin-fixed, paraffin-embedded) tissue as described below.

1. Set 2 water baths or heat blocks at 37ºC and 50°C, respectively.
2. Place 1–8 sections of 5–15 µm thick with a tissue surface area of 20–50 mm^2 (no more than 20 mg tissue) in a sterile microcentrifuge tube.
3. Add 1 mL CitriSolv™ Clearing Agent (Fisher cat. no. 22-143-975) to the sample and vortex vigorously for a few seconds.

   CitriSolv™ Clearing Agent is a biodegradable alternative to xylene for paraffin extraction.

   Note: You may also use xylene instead of CitriSolv™. Use appropriate precautions while using xylene and dispose of xylene in compliance with established institutional guidelines.
4. Centrifuge at maximum speed for 3 minutes at room temperature to pellet the tissue. Carefully remove the supernatant without disturbing the pellet.
5. Add 1 mL 96–100% ethanol and vortex to resuspend the tissue pellet.
6. Centrifuge at maximum speed for 3 minutes at room temperature to pellet the tissue. Carefully remove the supernatant without disturbing the pellet.
7. Repeat ethanol extraction (Steps 5–6) once more.
8. Incubate the tubes with lid open at 37ºC for 5–10 minutes to evaporate any residual ethanol.
9. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit). Mix well by brief vortexing.

   Note: When processing multiple samples, you may prepare a master Digestion Buffer Mix by mixing 180 µL Digestion Buffer and 20 µL Proteinase K for each sample.
10. Incubate at 50°C for 3 hours to overnight.
11. Centrifuge the lysate at maximum speed for 3 minutes at room temperature to remove any particulate materials. Transfer lysate to a new, sterile microcentrifuge tube.
12. Add 20 µL RNase A (supplied in the kit) to the lysate, mix well by brief vortexing, and incubate at room temperature for 2 minutes.
13. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and mix well by brief vortexing.
14. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.

    Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
15. Proceed immediately to **Binding DNA** (page 23).

Oragene™ Preserved Saliva

Process Oragene™ preserved saliva as described below.

Note: If you are processing >200 µL Oragene™ sample, you need to purchase additional PureLink® Genomic Lysis/Binding Buffer and Proteinase K (page 43).

Saliva Collection

Collect and preserve saliva as described by the Oragene™ device manufacturer. If the saliva sample is collected immediately prior to purification, incubate the sample at 50ºC for 1 hour before starting the protocol. Otherwise, an overnight incubation at room temperature in the Oragene™ device is sufficient to release and preserve genomic DNA.

Up to 1 mL Oragene™ Sample

1. Set a water bath or heat block at 55°C.
2. Transfer 1 volume of saliva mixture from Oragene™ self-collection device into an appropriate tube and mix with 1 volume of PureLink® Genomic Lysis/Binding Buffer. Choose an appropriate tube that can hold three times your sample volume to allow for reagent additions.

   For example, to 200 µL Oragene™ sample, add 200 µL PureLink® Genomic Lysis/Binding Buffer.
3. Incubate at 55ºC for 10 minutes.
4. Add 1 sample volume of 96–100% ethanol and mix well by brief vortexing.

   For example, if you used 200 µL Oragene™ sample, add 200 µL 96–100% ethanol.
5. Proceed immediately to **Binding DNA** (page 23).

4 mL Oragene™ Sample

1. Set a water bath or heat block at 55–65°C.
2. To the entire ~4 mL Oragene™ sample, add 400 µL 3 M sodium acetate (pH 5–5.5) and 2.8 mL isopropanol. Mix well by brief vortexing.
3. Centrifuge at >12,000 × *g* for 10 minutes at room temperature. Discard the supernatant.
4. Resuspend the pellet in 300 µL PBS (or 10 mM Tris, pH 8.0), and add 300 µL PureLink® Genomic Lysis/Binding Buffer.
5. Incubate at 55–65ºC for 5–10 minutes to solubilize the pellet containing cell debris and nucleic acid.
6. Add 300 µL 96–100% ethanol and mix well by brief vortexing.
7. Proceed immediately to **Binding DNA** (page 23).

Guidelines for Lysate Protocol Development

If none of the lysate preparation protocols described in this manual match the type or size of your sample, use the following guidelines to develop your own lysate preparation protocol.

- Lyse the sample using the PureLink® Genomic Digestion Buffer and Proteinase K supplied with the kit or use specialized lysis buffer or protocols to perform lysis. You may need to optimize lysis conditions prior to DNA purification to obtain the best results for your specific sample.
- Mix the sample with PureLink® Genomic Binding Buffer and 96–100% ethanol prior to loading the sample onto the column. Always maintain a ratio of 1:1:1 for Sample/Digestion Buffer:Binding Buffer:Ethanol to obtain optimal DNA binding.

A general protocol for lysate preparation can be as follows:

1. For cells, harvest cells and resuspend cell pellet in 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K. Incubate at 55°C until lysis is complete.

   For tissues, start with a small amount of minced tissue and add 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K to the sample and mix well. Incubate at 55°C until lysis is complete.

   Based on the results obtained using this lysis protocol, you may need to optimize the lysis protocol using different buffers or increasing the amount and time of Proteinase K digestion.

   If you already have a lysate, proceed to Step 2.
2. Add 20 µL RNase A (supplied with the kit). Incubate at room temperature for 2 minutes.
3. Centrifuge the lysate at maximum speed for 5 minutes at room temperature to remove any particulate material, if needed.
4. Transfer the supernatant to a fresh microcentrifuge tube. Add 200 µL PureLink® Genomic Binding Buffer supplied with the kit to the lysate. Mix well by vortexing to yield a homogenous solution.
5. Add 200 µL 96–100% ethanol to the lysate. Mix well by vortexing for 5 seconds to yield a homogenous solution.
6. Proceed to **Binding DNA**, page 23.

Purification Procedure Using Spin Columns

Introduction

The purification procedure is designed for purifying genomic DNA using a spin column-based centrifugation procedure in a total time of **10–15 minutes**.

Materials Needed

Components supplied by the user

- Lysates prepared as described on pages 16–21
- Sterile, DNase-free 1.5-mL microcentrifuge tubes for elution
- Microcentrifuge capable of centrifuging >10,000 × g
- Optional: sterile water, pH 7.0–8.5, if you are using water for elution

Components supplied with the Kit

- PureLink® Genomic Wash Buffers 1 and 2
- PureLink® Genomic Elution Buffer
- PureLink® Spin Columns in Collection Tubes
- PureLink® Collection Tubes

Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
- Review **Elution Parameters** on page 13 to determine the suitable elution volume for your requirements
- Perform a 1 minute incubation step with PureLink® Genomic Elution Buffer
- Be sure to perform the recommended wash steps to obtain the best results
- If you are using water for elution, always use sterile water, pH 7.0–8.5

Before Starting

Add 96–100% ethanol to PureLink® Genomic Wash Buffer 1 and PureLink® Genomic Wash Buffer 2 according to instructions on each label. Mix well. Mark on the labels that ethanol is added. Store both wash buffers with ethanol at room temperature.

Binding DNA

1. Remove a PureLink® Spin Column in a Collection Tube from the package.
2. Add the lysate (~640 µL) prepared with PureLink® Genomic Lysis/Binding Buffer and ethanol to the PureLink® Spin Column.
3. Centrifuge the column at 10,000 × *g* for 1 minute at room temperature.

   Note: If you are processing >200 µL starting material such as blood, buccal swabs, or Oragene™ preserved saliva, you need to perform multiple loading of the lysate by transferring any remaining lysate to the same PureLink® Spin Column (above) and centrifuge at 10,000 × *g* for 1 minute.
4. Discard the collection tube and place the spin column into a clean PureLink® Collection Tube supplied with the kit.
5. Proceed to **Washing DNA**, page 24.

Purification Procedure Using Spin Columns, Continued

Washing DNA

1. Add 500 µL Wash Buffer 1 prepared with ethanol (page 23) to the column.
2. Centrifuge column at room temperature at 10,000 × *g* for 1 minute.
3. Discard the collection tube and place the spin column into a clean PureLink® collection tube supplied with the kit.
4. Add 500 µL Wash Buffer 2 prepared with ethanol (page 23) to the column.
5. Centrifuge the column at maximum speed for 3 minutes at room temperature. Discard collection tube.
6. Proceed to **Eluting DNA**.

Eluting DNA

1. Place the spin column in a sterile 1.5-mL microcentrifuge tube.
2. Add 25–200 µL of PureLink® Genomic Elution Buffer to the column. See **Elution Parameters** (page 13) to choose the suitable elution volume for your needs.
3. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperature. *The tube contains purified genomic DNA.*
4. To recover more DNA, perform a second elution step using the same elution buffer volume as first elution in another sterile, 1.5-mL microcentrifuge tube.
5. Centrifuge the column at maximum speed for 1.5 minutes at room temperature.

   The tube contains purified DNA. Remove and discard the column.

Storing DNA

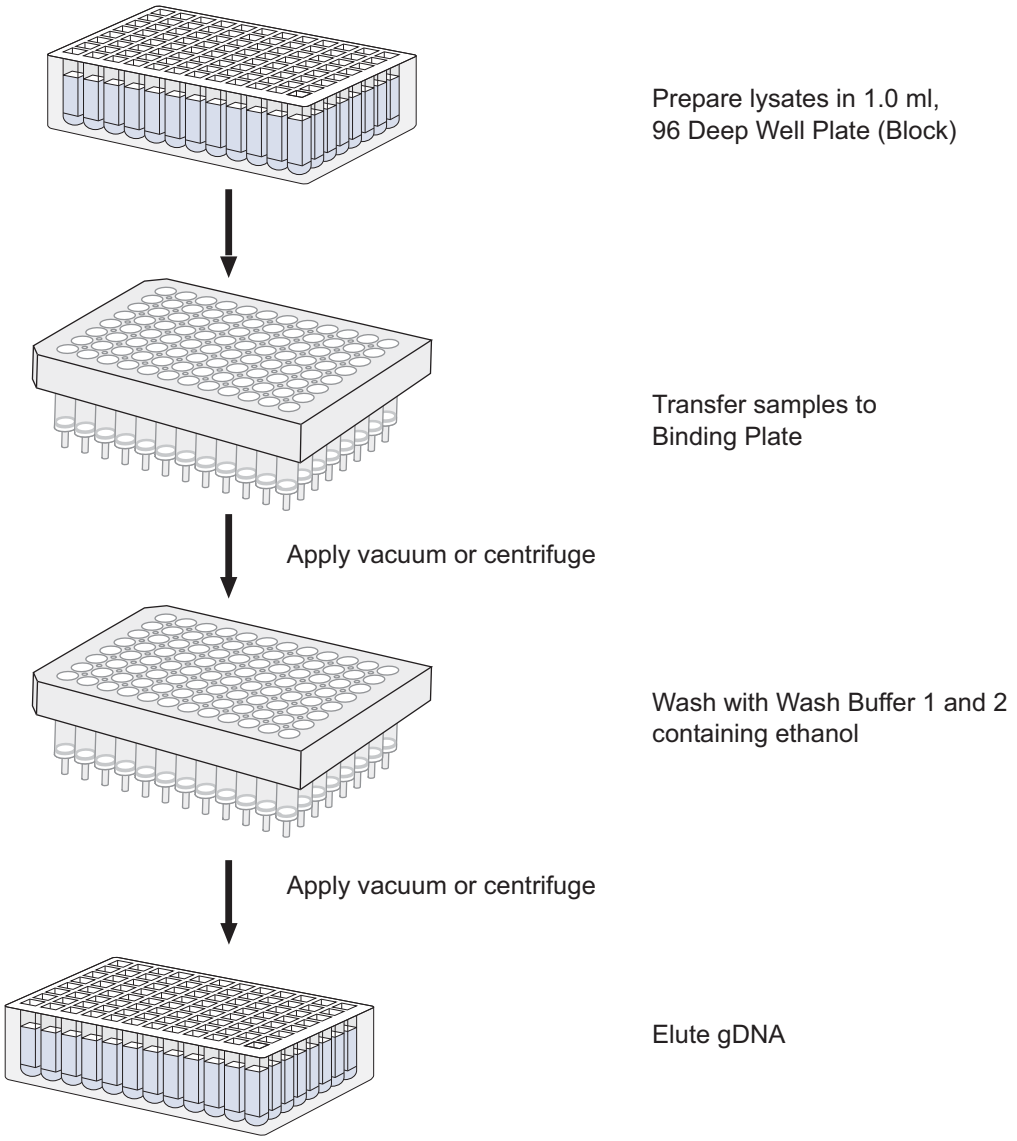
- Store the purified DNA at –20°C or use DNA for the desired downstream application.
- For long-term storage, store the purified DNA in PureLink® Genomic Elution Buffer at –20°C as DNA stored in water is subject to acid hydrolysis.
- To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage.

Purification Procedure Using 96 Kit

Experimental Overview

Introduction

The procedure for purifying genomic DNA using the PureLink® 96 Genomic DNA Kit is illustrated in the following flow chart.



Preparing Lysates–96 Kit

Introduction

Instructions for preparing lysates from mammalian cells and tissues, mouse tail, blood, buccal swabs, bacteria, yeast, FFPE tissues, and Oragene™ preserved saliva are described in the following sections.

To obtain high-quality genomic DNA, follow the guidelines recommended on page 11.

Important

Make sure there is no precipitate visible in PureLink® Genomic Digestion Buffer or PureLink® Genomic Lysis/Binding Buffer. If any precipitate is visible in the buffers, warm the buffers at 37°C for 3–5 minutes and mix well to dissolve the precipitate before use.

Materials Needed

Components supplied by the user

- 96–100% ethanol
- Sample for DNA isolation (see page 12 for recommended starting amount)
- Phosphate Buffered Saline (PBS) for mammalian cell lysate (page 43)
- Lysozyme and Lysozyme Digestion Buffer (25 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) for bacterial cell lysate
- Zymolase Buffer (1 M sorbitol, 10 mM sodium EDTA, 14 mM β-mercaptoethanol) and Zymolase (lyticase) enzyme for yeast lysate
- CitriSolv™ Clearing Agent (Fisher cat. no. 22-143-975) or xylene for FFPE tissue
- 3 M sodium acetate (pH 5–5.5) and 2.8 mL isopropanol for Oragene™ samples
- Water baths or heat blocks

Components supplied with the Kit

- PureLink® Genomic Lysis/Binding Buffer
- PureLink® Genomic Digestion Buffer
- Proteinase K (20 mg/mL)
- RNase A (20 mg/mL)
- 96 Deep Well Plates
- Foil Tape

Note

Do not reuse the Foil Tape during lysate preparation steps. After use, discard the Foil Tape and use a fresh Foil Tape for the next step. Sufficient Foil Tape is included in the kit. Additional Foil Tape is also available separately, see page 43 for ordering information.

Mammalian Cells and Blood Lysate

Use the following protocol to prepare lysate from mammalian cells and blood samples.

1. Set a water bath or heat block at 55°C.
2. Add 20 µL Proteinase K (supplied with the kit) to each well of a 96 Deep Well Plate.
3. Process cells or blood samples:
   - For adherent cells (≤5 × 10^6 cells), remove the growth medium from the culture plate and harvest cells by trypisinization or a method of choice. Resuspend the cells in 200 µL PBS.
   - For suspension cells (≤5 × 10^6 cells), harvest cells by centrifugation at 250 × *g* for 5 minutes. Remove the growth medium. Resuspend the cells in 200 µL PBS.
   - To a sterile microtiter plate, add up to 200 µL fresh or frozen blood sample (if using <200 µL blood sample, adjust the sample volume to 200 µL using PBS).
4. Transfer 200 µL cells or blood in PBS to each well of a 96 Deep Well Plate containing Proteinase K from Step 2.
5. Add 20 µL RNase A (supplied with the kit) to the sample. Seal the plate with Foil Tape. Mix well by brief vortexing, and incubate at room temperature for 2 minutes.
6. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and seal the plate with Foil Tape. Mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Incubate at 55°C for 10 minutes to promote protein digestion.
8. Add 200 µL 96–100% ethanol to the lysate and seal the plate with Foil Tape. Mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Mammalian Tissue and Mouse/Rat Tail Lysate

Use the following protocol to prepare lysate from mammalian tissues and mouse/rat tails.

1. Set a water bath or heat block at 55°C.
2. Place the following amount of mammalian tissue or tail into each well of a 96 Deep Well Plate:
   - Up to 25 mg of minced mammalian tissue
   - Up to 10 mg minced spleen tissue
   - 1 cm mouse or 0.5 cm rat tail clip
3. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit) to each well of the 96 Deep Well Plate. Ensure the tissue is completely immersed in the buffer mix. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Digestion Buffer Mix by mixing 180 µL Digestion Buffer and 20 µL Proteinase K for each sample.
4. Incubate at 55°C with occasional vortexing until lysis is complete (1–4 hours). For mouse tails or larger tissue pieces, you may perform overnight digestion.
5. Centrifuge the lysate at maximum speed for 3 minutes at room temperature to remove any particulate materials.
6. Add 20 µL RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
7. Add 200 µL PureLink® Genomic Lysis/Binding Buffer and seal the plate with Foil Tape. Mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
8. Add 200 µL 96–100% ethanol to the lysate and seal the plate with Foil Tape. Mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Blood Spots

Use the following protocol to prepare lysate from dried blood spots.

1. Set a water bath or heat block at 55°C.
2. Place 2–5 punches of dried blood spot (2–3 mm in size) in a well of a 96 Deep Well Plate.
3. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit) to each well. Ensure the pieces are completely immersed in buffer. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
4. Incubate at 55°C with occasional vortexing for 30 minutes.
5. Centrifuge the sample at maximum speed for 2–3 minutes at room temperature to pellet paper fibers. Transfer samples to a clean, 96 Deep Well Plate.
6. Add 20 µL RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
7. Add 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal plate with the Foil Tape and mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
8. Add 200 µL 96–100% ethanol to the lysate. Seal plate with the Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Gram Negative Bacterial Cell Lysate

Use the following protocol to prepare Gram negative bacterial cell lysate.

1. Set a water bath or heat block at 55°C.
2. Harvest up to 2 × 10^9 Gram negative (~1 mL of overnight *E. coli* culture) bacteria in a 96 Deep Well Plate by centrifugation at ≥2250 × *g* for 10 minutes.
3. Resuspend the cell pellet in 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K (supplied with the kit) to lyse the cells. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
4. Incubate the tube at 55°C with occasional vortexing until lysis is complete (30 minutes to up to 4 hours).
5. Add 20 µL RNase A (supplied with the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
6. Add 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal the plate with Foil Tape and mix well by vortexing to obtain a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Add 200 µL 96–100% ethanol to the lysate. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
8. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Gram Positive Bacterial Cell Lysate

Use the following protocol to prepare Gram positive bacterial cell lysate.

1. Set two water baths or heat blocks at 37ºC and 55°C, respectively.
2. Prepare Lysozyme Digestion Buffer (see recipe on page 26). To ~ 200 µL Lysozyme Digestion Buffer/sample, add **fresh** Lysozyme to obtain a final Lysozyme concentration of 20 mg/mL.
3. Harvest up to 2 × 10^9 Gram positive cells in a 96 Deep Well Plate by centrifugation at ≥2250 × *g* for 10 minutes.
4. Resuspend the cell pellet in 180 µL Lysozyme Digestion Buffer with Lysozyme from Step 2. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
5. Incubate at 37ºC for 30 minutes.
6. Add 20 µL Proteinase K and 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Incubate at 55°C for 30 minutes.
8. Add 200 µL 96–100% ethanol to the lysate. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Yeast Cells Lysate

Use the following protocol to prepare lysate from yeast cells.

1. Set 2 water baths or heat blocks at 37°C and 55°C, respectively.
2. Prepare **fresh** Zymolase Buffer (see recipe on page 26). You need 500 µL buffer per sample.
3. Harvest up to 5×10^7 yeast cells by centrifugation in a 96 Deep Well Plate.
4. Resuspend the cell pellet in 500 µL Zymolase Buffer. Add 15 units Zymolase (lyticase) enzyme and incubate at 37°C for 1 hour to generate spheroplasts.
5. Centrifuge at 3000 × *g* for 10 minutes at room temperature to pellet the spheroplasts.
6. Resuspend the spheroplasts in 180 µL PureLink® Genomic Digestion Buffer. Add 20 µL Proteinase K (supplied with the kit). Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
7. Incubate at 55°C for 45 minutes.
8. Add 20 µL RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
9. Add 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
10. Add 200 µL 96–100% ethanol to the lysate. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

    Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
11. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

FFPE Tissue Lysate

Use the following protocol to prepare lysates from FFPE (formalin-fixed, paraffin-embedded) tissue.

1. Set 2 water baths or heat blocks at 37ºC and 50°C, respectively.
2. Place 1–8 sections of 5–15 µm thick with a tissue surface area of 20–50 mm^2 (no more than 20 mg tissue) into each well of a 96 Deep Well Plate.
3. Add 1 mL CitriSolv™ Clearing Agent (Fisher cat. no. 22-143-975) to the sample. Seal the plate with Foil Tape and vortex vigorously for a few seconds.

   CitriSolv™ Clearing Agent is a biodegradable alternative to xylene for paraffin extraction.

   Note: You may also use xylene instead of CitriSolv™. Use appropriate precautions while using xylene and dispose of xylene in compliance with established institutional guidelines.
4. Centrifuge at maximum speed for 3 minutes at room temperature to pellet the tissue. Carefully remove the supernatant without disturbing the pellet.
5. Add 1 mL 96–100% ethanol. Seal the plate with Foil Tape and vortex to resuspend the tissue pellet.
6. Centrifuge at maximum speed for 3 minutes at room temperature to pellet the tissue. Carefully remove the supernatant without disturbing the pellet.
7. Repeat ethanol extraction (Steps 5–6) once more.
8. Incubate the plate **without the seal** at 37ºC for 5–10 minutes to evaporate any residual ethanol.
9. Add 180 µL PureLink® Genomic Digestion Buffer and 20 µL Proteinase K (supplied with the kit). Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

   Note: When processing multiple samples, you may prepare a master Digestion Buffer Mix by mixing 180 µL Digestion Buffer and 20 µL Proteinase K for each sample.
10. Incubate at 50°C for 3 hours to overnight.
11. Centrifuge the lysate at maximum speed for 3 minutes at room temperature to remove any particulate materials.
12. Add 20 µL RNase A (supplied in the kit) to the lysate and seal the plate with Foil Tape. Mix well by vortexing and incubate at room temperature for 2 minutes.
13. Add 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
14. Add 200 µL 96–100% ethanol to the lysate. Seal the plate with Foil Tape and mix well by vortexing to yield a homogenous solution. Briefly centrifuge the plate to collect any lysate from the Foil Tape.

    Note: When processing multiple samples, you may prepare a master Buffer/ethanol Mix by mixing 200 µL Lysis/Binding Buffer and 200 µL 96–100% ethanol for each sample.
15. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Human Buccal Swab Lysate

Use the following protocol to prepare lysate from human buccal cell swabs.

1. Set a water bath or heat block at 55°C.
2. Place the buccal swab in a sterile, 2 mL microcentrifuge tube. Add 400 µL (for cotton and Dacron swab) or 600 µL (for Omni Swab) PBS to the sample.
3. Add 20 µL Proteinase K into the wells of a 96 Deep Well Plate.
4. Transfer 200 µL swab lysate to 96 Deep Well Plate containing Proteinase K (Step 3).
5. Add 200 µL PureLink® Genomic Lysis/Binding Buffer to the lysate. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
6. Incubate at 55°C for at least 10 minutes. Keep the plate covered with Foil Tape during the incubation.
7. Centrifuge briefly to collect any lysate from the Foil Tape.
8. Add 200 µL 96–100% ethanol to the tube. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
9. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Oragene™ Preserved Saliva

Process up to 200 µL Oragene™ preserved saliva as described in the following protocol.

Saliva Collection

Collect and preserve saliva as described by the Oragene™ device manufacturer. If the saliva sample is collected immediately prior to purification, incubate the sample at 50°C for 1 hour before starting the protocol. Otherwise, an overnight incubation at room temperature in the Oragene™ device is sufficient to release and preserve genomic DNA.

1. Set a water bath or heat block at 55°C.
2. Transfer 200 µL of saliva mixture from Oragene™ self-collection device into each well of a 96 Deep Well Plate and mix with 200 µL PureLink® Genomic Lysis/Binding Buffer. Seal plate with a Foil Tape.
3. Incubate at 55°C for 10 minutes.
4. Add 200 µL 96–100% ethanol. Seal the plate with Foil Tape and mix well by vortexing. Briefly centrifuge the plate to collect any lysate from the Foil Tape.
5. Proceed immediately to **Purification Using Centrifugation** (page 36) or **Vacuum Manifold** (page 37).

Purification Procedure Using 96-Well Plates

Introduction

The purification procedure is designed for purifying genomic DNA with 96-well plates with a vacuum manifold or centrifuge in a total time of **30–45 minutes**.

Follow the recommendations below to obtain the best results:

- Perform all centrifugation steps at room temperature
- Review **Elution Parameters** on page 13 to determine the suitable elution volume for your requirements
- Perform a 1 minute incubation step with PureLink® Genomic Elution Buffer
- Be sure to perform the recommended wash steps to obtain the best results
- If you are using water for elution, always use sterile water, pH 7.0–8.5
- Use the recommended vacuum pressure

Instrument Compatibility for 96-Well Plates

The PureLink® gDNA Binding Plates are compatible with the following instruments:

- **Vacuum Manifold:** The manifold must accommodate the PureLink® Plates and be capable of collecting the filtrate (e.g., PureLink® Vacuum Manifold, page 43, UniVac™ 3 Vacuum Manifold System from Whatman, or QIAvac 96 from Qiagen).
- **Centrifuge:** Must be capable of centrifuging 96-well plates at ≥2250 × g, and accommodate a 5.75 cm microtiter plate stack.
- **Automated Liquid Handling Workstation:** The workstation must be equipped with a vacuum manifold and a vacuum source. The PureLink® gDNA Binding Plate is compatible for use on the Biomek® FX, Tecan Freedom EVO™, and BioRobot® Workstations.

  Note: If you are using the BioRobot® Workstation, you can perform elution using centrifugation or vacuum manifold as described on page 38.

Calibrating Vacuum for Use with 96-Well Plates

We recommend using a vacuum pressure of –6 to –12 inches Hg (–200 to –400 mbar or –150 to –300 mm Hg) to obtain the best results.

Using higher vacuum pressure than the recommended pressure may cause sample splattering or inefficient DNA binding, while using lower vacuum pressure will affect the elution resulting in lower recovery.

To check the vacuum pressure:

1. Place an unused PureLink® gDNA Binding Plate on top of the vacuum manifold and seal the plate with Foil Tape.
2. Apply vacuum and check the vacuum pressure on the vacuum regulator (usually attached to the manifold or a vacuum pump).
3. Adjust the vacuum pressure on the regulator to obtain the recommended pressure of –6 to –12 inches Hg.

   Note: During purification the vacuum pressure may exceed the recommended value.

Purification Procedure Using 96-Well Plates, Continued

Materials Needed

Components supplied by the user

- Lysates prepared as described on pages 27–33
- Vacuum manifold and a vacuum pump for 96-well plates (producing pressure of –6 to –12 inches Hg or –200 to –400 mbar or –150 to –300 mm Hg) or automated liquid handling workstations

  or
- Centrifuge with a swinging bucket rotor with 96-well plate carriers that have a plate height clearance of ~5.75 cm, and capable of centrifuging at ≥2250 × *g* at 25°C
- Optional: sterile, DNase-free 1.5 cm standard microtiter plates for elution
- Optional: sterile water, pH 7.0–8.5, if you are using water for elution

Components supplied with the Kit

- PureLink® Genomic Wash Buffers 1 and 2
- PureLink® Genomic Elution Buffer
- PureLink® gDNA Binding and Wash Plates
- 96 Deep Well Plates and Foil Tape

Note

- The purified gDNA can be eluted into 96 Deep Well Plates (supplied with the kit) or standard 1.5 cm microtiter plates (not supplied).
- The 96 Deep Well Plates can be reused. However, for elution, we recommend that you use a clean, **unused** 96 Deep Well Plate.
- If you are using automated liquid handling workstations for purification, you may need additional buffers depending on your type of workstation. Individual PureLink® Genomic Buffers are available, see page 43 for ordering information.

PureLink® Genomic DNA Wash Plate

The PureLink® Genomic DNA Wash Plate is designed to prevent cross contamination of the silica membrane when used with the PureLink® Vacuum Manifold or equivalent. The PureLink® Genomic DNA Wash Plate is a microtiter plate that is open on both sides allowing free flow of buffers.

The vacuum manifold is assembled with the wash plate placed underneath the binding plate. During washing, 96 separate channels are formed when the outlets on the binding plate protrude into the wash plate wells. This prevents any spraying of wash buffer onto the binding plate thereby reducing cross contamination and ethanol carry over. The wash plate is removed and discarded after the washing steps.

Before Starting

Add 96–100% ethanol to PureLink® Genomic Wash Buffer 1 and PureLink® Genomic Wash Buffer 2 according to instructions on each label. Mix well. Mark on the labels that ethanol is added. Store both wash buffers with ethanol at room temperature.

Purification Using Centrifugation

1. Assemble the PureLink® gDNA Binding Plate onto a new or used 96 Deep Well Plate supplied with the kit. Ensure that the nozzles of the Binding Plate extend into the wells of the 96 Deep Well Plate.
2. Transfer each lysate (~640 µL) to a well of the PureLink® gDNA Binding Plate using a multichannel pipettor. Cover any unused wells with Foil Tape.
3. Centrifuge the stacked plates at ≥2250 × *g* for 5–10 minutes.
4. Discard the flow through and reassemble the PureLink® gDNA Binding Plate onto the used 96 Deep Well Plate.
5. Add 500 µL Wash Buffer 1 prepared with ethanol (page 35) into each well of the PureLink® gDNA Binding Plate.
6. Centrifuge the stacked plates at ≥2250 × *g* for 5–10 minutes.
7. Discard the flow through and reassemble the plate stack.
8. Add 500 µL Wash Buffer 2 prepared with ethanol (page 35) into each well of the PureLink® gDNA Binding Plate.
9. Centrifuge the stacked plates at ≥2250 × *g* for 15 minutes.

   Note: To ensure the complete drying of the membrane, do not seal the plate.
10. Discard the flow through and reassemble the PureLink® gDNA Binding Plate onto a **new** 96 Deep Well Plate supplied with the kit.
11. Add 50–200 µL PureLink® Genomic Elution Buffer to the center of the membrane in each well and incubate the plate for 1 minute at room temperature.

    Note: Review **Elution Parameters** on page 13 to choose a suitable elution volume for your needs.

    Centrifuge the stacked plates at ≥2250 × *g* for 3 minutes. *The purified gDNA is eluted in the Deep Well Plate.*
12. If desired, perform a second elution to increase recovery which lowers the overall concentration.
13. Use the purified gDNA for the desired downstream application. To store the purified gDNA, cover the wells with a Foil Tape, and store at 4ºC for short-term or –20°C for long-term storage.

Purification Using Vacuum Manifold

1. Assemble the vacuum manifold as per the manufacturer's instructions.

   Brief instructions for assembling the PureLink® Vacuum Manifold are described below. Refer to the manual supplied with the manifold for details.

   a. Insert the waste tray into the manifold base.

   b. Insert one set of MTP/Multi96 plate adaptors into each side slot of the manifold base.

   c. Place the PureLink® Genomic DNA Wash Plate over adaptors to prevent any cross contamination. See page 35 for a description of the Wash Plate.

   d. Place the manifold lid on the manifold base to close the manifold.

   Note: The Wash Plate is not needed when using the UniVac™ 3 Vacuum Manifold (Whatman). To use the Wash Plate with QiaVac 96 Vacuum Manifold (Qiagen), you need the PureLink® Adaptor Frame (page 43).

2. Place the PureLink® gDNA Binding Plate onto the vacuum manifold designed to hold a 96-well plate.
3. Transfer the lysates (~640 µL) from each well of the Deep-Well Plate to a fresh well in the Binding Plate. Cover unused wells with Foil Tape.
4. Apply vacuum at room temperature until the lysate completely passes through the filter plate and release vacuum.
5. Add 1 mL Wash Buffer 1 prepared with ethanol (page 35) into each well of the Binding Plate.
6. Apply vacuum for 2 minutes at room temperature. Release vacuum.
7. Add 1 mL Wash Buffer 2 prepared with ethanol (page 35) into each well of the Binding Plate.
8. Apply vacuum for 2 minutes at room temperature. Release vacuum.
9. Disassemble the manifold to remove and discard the wash plate. Tap the Binding Plate on paper towels to remove any residual Wash Buffer from the nozzles. Reassemble the manifold with the binding plate.
10. Apply vacuum for 10 minutes at room temperature to allow membrane drying. Release vacuum.
11. Disassemble the manifold to remove the waste tray. Discard the waste tray contents.
12. Assemble the vacuum manifold with a Deep Well Plate or 1.5 cm microtiter plate for elution as per the manufacturer's instructions. See next page for brief instructions on assembling various vacuum manifolds with different types of adaptors and elution plates based on the type of plate used for elution.
13. Place the PureLink® gDNA Binding Plate onto the vacuum manifold.

Purification Using Vacuum Manifold, continued

14. Add 100–200 µL of PureLink® gDNA Elution Buffer to the center of the membrane in each well of the Binding Plate and incubate the plate for 1 minute at room temperature. **Note:** Review **Elution Parameters** (page 13) to choose a suitable elution volume.
15. Apply vacuum for 2 minutes at room temperature. Release vacuum. Disassemble the vacuum manifold to remove the elution plate. *The purified gDNA is eluted into the elution plate.*

Use the purified gDNA for the desired downstream application. To store the purified gDNA, cover the wells with a Foil Tape, and store at 4ºC for short-term or –20°C for long-term storage.

Assembling Vacuum Manifold for Elution

The purified gDNA is eluted from the vacuum manifold in a 96 Deep Well Plate (supplied with the kit) or a standard 1.5-cm microtiter plate (not supplied). Based on your vacuum manifold, you may need to use adaptors with the elution plate to ensure the elution plate is aligned with Binding Plate.

PureLink® Vacuum Manifold (Life Technologies)

Brief instructions for assembling the PureLink® Vacuum Manifold for elution are described below. Refer to the manual supplied with the manifold for details.

For 96 Deep Well Plate

1. Insert one set of Round well adaptors followed by one set of Microtube Rack adaptors into each side slot of the manifold base.
2. Place the 96 Deep Well Plate supplied with the kit on the adaptors.
3. Place the manifold lid on the manifold base to close the manifold.

For 1.5-cm Microtiter Plate

1. Insert one set of MTP/Multi96 plate adaptors into each side slot of the manifold base.
2. Place the 1.5-cm microtiter plate (not supplied) on the adaptor.
3. Place the manifold lid on the manifold base to close the manifold.

   Note: Do not use >100 µL elution volume when using the microtiter plate for elution to prevent any contact of the Binding Plate nozzles with the eluate.

UniVac™ 3 Vacuum Manifold (Whatman)

Perform elution directly into the 96 Deep Well Plates supplied with the kit. There is no need for any adaptors. Elution into 1.5 cm microtiter plates or 1.5-mL racked microtubes is not recommended.

QiaVac 96 Vacuum Manifold (Qiagen)

- For elution into 96 Deep Well Plates supplied with the kit, use the manifold with a suitable adaptor of 20–22 mm height.
- For elution into 1.5-cm microtiter plates (not supplied), use the PureLink® Adaptor Frame from Life Technologies (page 43) with the manifold. Be sure to use vacuum pressure of up to –200 mbar to prevent any splashing of the eluate.
- For elution into 1.5-mL racked microtubes, there is no need for any adaptors.

Results and Troubleshooting

Analyzing DNA Yield and Quality

DNA Yield

After purification with PureLink® Genomic DNA Purification Kit, the yield of purified DNA can be estimated by UV absorbance at 260 nm or Quant-iT™ DNA Assay Kits.

UV Absorbance

1. Measure the A_{260} of the solution using a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5–8.5.
2. Calculate the amount of DNA using formula:

DNA (µg) = A_{260} × 50 µg/(1 A_{260} × 1 mL) × dilution factor × total sample volume (mL)

For DNA, A_{260} = 1 for a 50 µg/mL solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ DNA Assay Kits

The Quant-iT™ DNA Assay Kits (page 43) provide a rapid, sensitive, and specific method for dsDNA quantitation with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

Each kit contains a state-of-the-art quantitation reagent and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microplate readers/fluorometers or the Qubit™ Fluorometer.

DNA Quality

Typically, DNA isolated using the PureLink® Genomic DNA Purification Kit has an A_{260}/A_{280} >1.80 when samples are diluted in Tris-HCl (pH 7.5) indicating that the DNA is reasonably clean of proteins that could interfere with downstream applications. Absence of contaminating RNA may be confirmed by agarose gel electrophoresis.

DNA Length

Genomic DNA isolated with the PureLink® Genomic DNA Purification Kit is usually in the size range of 20–50 kb. To determine the exact size of DNA, perform Pulse-Field Gel Electrophoresis (PFGE) on an agarose gel.

The DNA isolated using the PureLink® Genomic DNA Kits is suitable for use in PFGE without ethanol precipitation or any additional steps. General guidelines for PFGE are described in this section. For details, refer to the manufacturer's recommendations.

For PFGE, load 20 µL (0.5–1 µg) purified DNA/lane in 10X BlueJuice™ Gel loading Buffer on a 1% agarose gel in 0.5X TBE using appropriate PFGE molecular weight DNA ladders. Perform electrophoresis at 6 V/cm for 15 hours at 14°C using a switch time of 1–7 seconds. The gel is stained with ethidium bromide after electrophoresis to visualize the DNA.

Expected Results

DNA Yield

The yield of genomic DNA obtained from various samples using the PureLink® Genomic DNA Mini Kits is listed below. The DNA quantitation was performed using UV absorbance at 260 nm. The yield is the total yield from 2 × 200 µL elutions.

Material	Amount	DNA Yield
E.coli cells	2×10^9	10–30 µg
HeLa cells	5×10^6	20–40 µg
293F cells	5×10^6	15–30 µg
Human Blood	200 µL	3–10 µg
Mouse Tail	1–1.2 cm	5–25 µg
Mouse Brain	25 mg	10–30 µg
Mouse Liver	25 mg	10–30 µg
Mouse Spleen	10 mg	10–40 µg

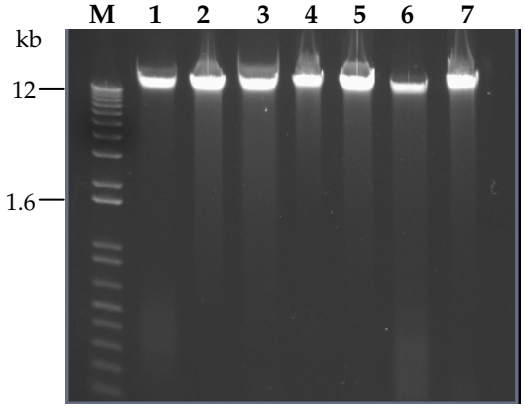
Note: The DNA yield varies with the sample and DNA content of the sample.

DNA Quality

Genomic DNA isolated from various samples was analyzed by agarose gel electrophoresis on a 1% E-Gel® agarose gel.

Samples on the gel are:

Lane M: 1 Kb Plus DNA Ladder
Lane 1: 200 ng DNA isolated from Gram positive bacteria (2×10^9 cells)
Lane 2: 200 ng DNA isolated from Gram negative bacteria, *E. coli* (2×10^9 cells)
Lane 3: 200 ng DNA isolated from human 293F (5×10^6 cells)
Lane 4: 200 ng DNA isolated from human whole blood (200 µL)
Lane 5: 200 ng DNA isolated from rat brain tissue (20 mg)
Lane 6: 200 ng DNA isolated from human saliva (200 µL of Oragene™ sample)
Lane 7: 200 ng DNA isolated from rat liver tissue (20 mg)



Troubleshooting

Introduction

Refer to the following table to troubleshoot any problems you may encounter with the PureLink® Genomic DNA Kits.

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	• Decrease the amount of starting material used. • Be sure to add Proteinase K during lysis. • For tissues, cut the tissue into smaller pieces and ensure the tissue is completely immersed in the Digestion Buffer to obtain optimal lysis. • If incomplete lysis is observed, increase the digestion time or amount of Proteinase K used for lysis.
	Poor quality of starting material	Be sure to use fresh sample and process immediately after collection or freeze the sample at –80°C or in liquid nitrogen. The yield and quality of DNA isolated is dependent on the type and age of the starting material.
	PureLink® Spin Column or Binding Plate is clogged	Make sure that the lysate is clear when the lysate is loaded on to the spin column or plate. Remove any particulate or viscous material by centrifugation prior to loading the lysate on to the spin column or plate.
	Incorrect binding conditions	• Be sure to add PureLink® Lysis/Binding Buffer and 96–100% ethanol to the lysate prior to loading the samples on the spin column or Binding Plate. Mix the sample properly with Binding Buffer and ethanol by vortexing. • Avoid overloading the column or plate.
	Ethanol not added to Wash Buffers 1 and 2	Be sure to add 96–100% ethanol to Wash Buffers 1 and 2 as indicated on the label.
	Incorrect elution conditions	• Add elution buffer and perform incubation for 1 minute with elution buffer before centrifugation. • To recover more DNA, perform a second elution step.
	DNA is sheared or degraded	• Avoid repeated freezing and thawing of samples to prevent any DNA damage. • Maintain a sterile environment while working to avoid any contamination from DNases.

Problem	Cause	Solution
Dark colored eluate or discolored membrane (mammalian tissue, mouse tails, or blood samples only)	Pigments from tissues or heme from blood bind to the silica matrix and co-elute with DNA	• Be sure to add ethanol to the lysate prior to loading the lysate on to the spin column or plate. The ethanol prevents the pigments from sticking on the silica matrix. • Perform centrifugation of the lysate at a higher speed and longer time prior to loading the lysate on to the column or plate. • If the problem persists, perform an additional wash step with 500 µL Wash Buffer 1 to obtain a total of two 500 µL wash steps with Wash Buffer 1 followed by a single 500 µL wash with Wash Buffer 2.
RNA contamination	Silica membrane binds total nucleic acid present in the sample	Perform RNase digestion step during sample preparation.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified DNA	Traces of ethanol from the Wash Buffer 2 can inhibit downstream enzymatic reactions. • To remove Wash Buffer 2 from spin columns, discard Wash Buffer 2 flow through. Place the spin column into the Wash Tube and centrifuge the spin column at maximum speed for 2–3 minutes to completely dry the column. • To remove any traces of Wash Buffer 2 from the Binding Plate and dry the membrane, centrifuge the plate stack at ≥2250 × *g* for 15 minutes or apply vacuum for 10 minutes. The plate can also be warmed at 70ºC for 10 minutes to evaporate any ethanol.
	Presence of salt in purified DNA	• Use the correct order of Wash Buffers for washing. Always wash with Wash Buffer 1 followed by washing with Wash Buffer 2. • Always maintain a ratio of 1:1:1 for Sample:Binding Buffer:Ethanol.
Low elution volume or sample cross-contamination	Incorrect vacuum pressure	• Make sure the vacuum manifold is sealed tightly and there is no leakage. A vacuum pressure of –6 to –12 inches Hg (–200 to –400 mbar or –150 to –300 mm Hg) is required for best results. • To avoid any cross contamination and ensure proper contact between the PureLink® Genomic Binding Plate and elution plate, raise the elution plate in the vacuum manifold using adaptors as described on page 38.

Appendix

Safety

WARNING! GENERAL CHEMICAL HANDLING. For every chemical, 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 **www.lifetechnologies.com/support**.

The PureLink® Genomic Lysis/Binding Buffer and Wash Buffer 1 contain guanidine hydrochloride. Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid wastes containing this product.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/%2029cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**.

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at:
www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Accessory Products

Additional Products

The following products are also available from Life Technologies. For more details on these products, visit **www.lifetechnologies.com** or contact Technical Support (page 45).

Product	Quantity	Catalog No.
PureLink® Genomic DNA Mini Kit	10 preps	K1820-00
PureLink® Genomic Digestion Buffer	70 mL	K1823-01
PureLink® Genomic Lysis/Binding Buffer	80 mL	K1823-02
PureLink® Genomic Wash Buffer 1	100 mL	K1823-03
PureLink® Genomic Wash Buffer 2	75 mL	K1823-04
PureLink® Genomic Elution Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)	160 mL	K1823-05
Proteinase K (20 mg/mL)	5 mL	25530-049
Foil Tape	50 pieces	12261-012
PureLink® Vacuum Manifold	1 each	K2110-01
PureLink® Vacuum Regulator	1 each	K2110-02
PureLink® Adaptor Frame	1 each	K2110-03
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Phosphate Buffered Saline (PBS), 1X	500 mL	10010-023

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentages and well formats for your convenience.

A large variety of DNA ladders is available from Life Technologies for sizing DNA.

For more details on these products, visit **www.lifetechnologies.com** or contact Technical Support (page 45).

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

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 found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Purchaser Notification

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Thermo SCIENTIFIC

PRODUCT INFORMATION

ThermoScientific DreamTaq Green DNA Polymerase

#EP0712 **500 U**

Lot: __ **Expiry Date: __**

Store at -20°C

Ordering Information

Component	DreamTaq DNA Polymerase, 5 U/µL	10X DreamTaq Green Buffer*
#EP0711	200 U	1.25 mL
#EP0712	500 U	2 × 1.25 mL
#EP0713	5 × 500 U	10 × 1.25 mL
#EP0714	20 × 500 U	40 × 1.25 mL

* includes 20 mM $MgCl_2$

www.thermoscientific.com/onebio

Description

Thermo Scientific DreamTaq Green DNA Polymerase is a combination of DreamTaq™ DNA Polymerase and 10X DreamTaq Green Buffer. DreamTaq™ DNA Polymerase is an enhanced Taq DNA Polymerase optimized for high throughput PCR applications. It ensures higher sensitivity, longer PCR products and higher yields compared to conventional Taq DNA polymerase. DreamTaq Green DNA Polymerase incorporates modified nucleotides, but is inhibited by dUTP.
The 10X DreamTaq Green Buffer includes a density reagent and two tracking dyes for direct loading of PCR products on a gel. The colored buffer does not interfere with PCR performance and is compatible with downstream applications such as DNA sequencing, phosphorylation, ligation and restriction digestion. For applications that require PCR product analysis by absorbance or fluorescence excitation, we recommend using the colorless 10X DreamTaq Buffer (#B65) or purifying the PCR product prior to analysis.

Features

- Save time – go directly from PCR to gel electrophoresis.
- High yields of PCR products with minimal optimization.
- Higher sensitivity compared to conventional *Taq* DNA Polymerase.
- Amplification of long targets (up to 6 kb from genomic DNA, up to 20 kb from viral DNA).
- Robust amplification of difficult templates.

Applications

- Routine PCR amplification of DNA fragments up to 6 kb from from genomic DNA and up to 20 kb from viral DNA.
- RT-PCR.
- Genotyping.
- Generation of PCR products for TA cloning.

Concentration

5 U/µL

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30 min at 70°C.
Enzyme activity is assayed in the following mixture: 67 mM Tris-HCl (pH 8.8 at 25°C), 6.7 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 50 mM NaCl, 0.1 mg/mL BSA, 0.75 mM activated calf thymus DNA, 0.2 mM of each dNTP, 0.4 MBq/mL [^{3}H]-dTTP.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 8.0), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% (v/v) Nonidet P40, 0.5% (v/v) Tween 20 and 50% (v/v) glycerol.

10X DreamTaq Green Buffer

A proprietary formulation which, in addition to the PCR buffer components, includes a density reagent and two tracking dyes for direct loading of PCR products on a gel. The 10X DreamTaq Green Buffer contains KCl and $(NH_4)_2SO_4$ at a ratio optimized for robust performance in PCR and includes $MgCl_2$ at a concentration of 20 mM.

Inhibition and Inactivation

- Inhibitors: ionic detergents (deoxycholate, sarkosyl and SDS) at concentrations higher than 0.06, 0.02 and 0.01%, respectively.
- Inactivated by phenol/chloroform extraction.

PROTOCOL

To set up parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers and DreamTaq DNA Polymerase. Prepare enaugh master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes and then add template DNA.

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

10X DreamTaq Green Buffer*	5 µL
dNTP Mix, 2 mM each (#R0241)	5 µL (0.2 mM of each)
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	10 pg - 1 µg
DreamTaq DNA Polymerase	1.25 U
Water, nuclease-free (#R0581)	to 50 µL
Total volume	50 µL

*10X DreamTaq Green Buffer contains 20 mm $MgCl_2$, which is optimal for most applications. If additional optimization is required, 25 mm $MgCl_2$ (#R0971) can be added to the master mix. The volume of water should be reduced accordingly.

Volumes of 25 mM $MgCl_2$, required for specific final $MgCl_2$ concentration:

Final concentration of $MgCl_2$	2 mM	2.5 mM	3 mM	4 mM
Volume of 25 mM $MgCl_2$ to be added for 50 µL reaction	0 µL	1 µL	2 µL	4 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. Place the reactions in a thermal cycler. Perform PCR using recommended thermal cycling conditions:

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	
Final Extension	72	5-15 min	1

* The recommended extension step is 1 min for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 min/kb.

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 use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Use PCR-certified reagents, including high quality water (e.g., Water, nuclease-free, #R0581).
- Always perform “no template control” (NTC) reactions to check for contamination.

DreamTaq DNA Polymerase does not incorporate dUTP, therefore it is not possible to perform carryover contamination prevention with UDG. For this application we recommend using *Taq* DNA Polymerase (#EP0401) or Thermo Scientific Maxima Hot Start *Taq* DNA polymerase (#EP0601).

GUIDELINES FOR PRIMER DESIGN

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

- PCR primers are generally 15-30 nucleotides long.
- 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.

(continued on reverse page)

- When designing degenerate primers, place at least 3 conservated 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.onebio.com to determine the number of extra bases required for efficient cleavage.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

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 specialized computer programs e.g., REviewer (www.thermoscientific.com/reviewer) is recommended 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 are suitable for template preparation 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, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol normally removes trace contaminants from DNA samples.

$MgCl_2$ concentration

DreamTaq Green DNA Polymerase is provided with an optimized 10X DreamTaq Green Buffer which includes $MgCl_2$ at a concentration of 20 mM. A final $MgCl_2$ concentration of 2 mM is generally ideal for PCR. The $MgCl_2$ concentration can be further increased up to 4 mM by the addition of 25 mM $MgCl_2$ (#R0971).

If the DNA samples contain EDTA or other metal chelators, the Mg^{2+} ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds 1 Mg^{2+}).

dNTPs

The recommended final concentration of each dNTP is 0.2 mM. In certain PCR applications, higher dNTP concentrations may be necessary. It is essential to have equal concentrations of all four nucleotides (dATP, dCTP, dGTP and dTTP) present in the reaction mixture.

To obtain a 0.2 mM concentration of each dNTP in the PCR mixture, please refer to the table below:

Volume of PCR mixture	dNTP Mix, 2 mM each (#R0241)	dNTP Mix, 10 mM each (#R0191)	dNTP Mix, 25 mM each (#R1121)
50 µL	5 µL	1 µL	0.4 µL
25 µL	2.5 µL	0.5 µL	0.2 µL
20 µL	2 µL	0.4 µL	0.16 µL

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.

CYCLING PARAMETERS

Initial DNA denaturation

It is essential to completely denature the template DNA at the beginning of the PCR run 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. For GC-rich templates this step should be prolonged up to 10 min. If a longer initial denaturation step is required, or if the DNA is denatured at a higher temperature, DreamTaq DNA Polymerase should be added after the initial denaturation step to avoid a decrease in its activity.

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. DNA denaturation can also be enhanced by the addition of either 10-15% glycerol, 10% DMSO, 5% formamide or 1-1.5 M betaine. The melting temperature of the primer-template complex decreases significantly in the presence of these reagents. Therefore, the annealing temperature has to be adjusted accordingly.

In addition, 10% DMSO and 5% formamide inhibit DNA polymerases by 50%. Thus, the amount of the enzyme in the reaction should be increased if these additives are used.

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. When additives which change the melting temperature of the primer-template complex are used (glycerol, DMSO, formamide and betaine), the annealing temperature must also be adjusted.

Extension

The optimal extension temperature for DreamTaq DNA Polymerase is 70-75°C. The recommended extension step is 1 min at 72°C for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 min/kb. For amplification of templates >6 kb a reduction of the extension temperature to 68°C is recommended to avoid enzyme inactivation during prolonged extension times.

Number of cycles

The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield.

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 additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product will be cloned into TA vectors (for instance, using Thermo Scientific InsTAclone PCR Cloning Kit (#K1213)), the final extension step may be prolonged to 30 min to ensure the complete 3'-dA tailing of the PCR product. If the PCR product will 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 conversion of covalently closed circular DNA to nicked DNA was detected after incubation of 10 units of DreamTaq Green DNA Polymerase with 1 µg of pUC19 DNA for 4 hours at 37°C.

Exodeoxyribonuclease Assay

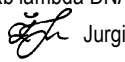
No degradation of DNA was observed after incubation of 1 µg lambda DNA/HindIII fragments with 10 units of DreamTaq Green DNA Polymerase for 4 hours at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 10 units of DreamTaq Green DNA Polymerase with 1 µg of [^{3}H]-RNA for 4 hours at 37°C.

Functional Assay

DreamTaq DNA Polymerase was tested for amplification of 956 bp single copy gene from human genomic DNA and for amplification of 20 kb lambda DNA fragment.

Quality authorized by:  Jurgita Žilinskienė

dNTP Set, molecular biology grade

Catalog Number R0181, R0182, R0186

Pub. No. MAN0013132 **Rev.** C.00

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

Contents and storage

Cat. No.	Contents	Amount	Storage
R0181	dATP, dCTP, dGTP, dTTP	4 x 0.25 mL, 100 mM	-25 °C to -15 °C
R0182	dATP, dCTP, dGTP, dTTP	4 x 1 mL, 100 mM	
R0186	dATP, dCTP, dGTP, dTTP	4 x 5 mL, 100 mM	

Description

The set consists of 100 mM aqueous solutions of dATP, dCTP, dGTP and dTTP each supplied in a separate vial. The nucleotide solutions are titrated to pH 7.3-7.5 with NaOH. Since the nucleotides are provided separately, the dNTP Set offers maximum flexibility in preparation of reaction mixes for different applications.

Applications

For use in PCR, real-time PCR, high fidelity and long PCR, LAMP-PCR, cDNA synthesis, RT-PCR, RDA, MDA, DNA labeling, and DNA sequencing.

General Characteristics

dATP $C_{10}H_{13}N_5O_{12}P_3Na_3$; MW = 557.2;
λ_{max}=259 nm; ε=15.4×10^3 M^{-1}cm^{-1} at pH 7.0;

dCTP $C_9H_{13}N_3O_{13}P_3Na_3$; MW = 533.1;
λ_{max}=271 nm; ε=9.1×10^3 M^{-1}cm^{-1} at pH 7.0.

dGTP $C_{10}H_{13}N_5O_{13}P_3Na_3$; MW = 573.2;
λ_{max}=253 nm; ε=13.7×10^3 M^{-1}cm^{-1} at pH 7.0.

dTTP $C_{10}H_{14}N_2O_{14}P_3Na_3$; MW = 548.1;
λ_{max}=267 nm; ε=9.6×10^3 M^{-1}cm^{-1} at pH 7.0.

Important Note

Mix well each dNTP solution prior to use.

Preparation of different concentration dNTP mixtures

dNTP mixture to be prepared	Volumes of dNTP Set, µL				Water, nuclease-free, µL	Total volume of dNTP mixture, µL
	100 mM dATP	100 mM dGTP	100 mM dCTP	100 mM dTTP		
2 mM of each dNTP	10	10	10	10	460	500
	100	100	100	100	4600	5000
	250	250	250	250	11500	12500
10 mM of each dNTP	10	10	10	10	60	100
	100	100	100	100	600	1000
	250	250	250	250	1500	2500
25 mM of each dNTP	10	10	10	10	–	40
	100	100	100	100	–	400
	250	250	250	250	–	1000

Getting 0.2 mM dNTP in PCR

Volume of PCR Mixture	dNTP Mixture to be added to PCR		
	2 mM	10 mM	25 mM
25 µL	2.5 µL	0.5 µL	0.2 µL
50 µL	5 µL	1 µL	0.4 µL
100 µL	10 µL	2 µL	0.8 µL

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**.

Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific DreamTaq Hot Start DNA Polymerase

Pub. No. MAN0015972
Rev. Date 29 July 2016 (Rev. A.00)

Lot: __ Expiry Date: __

Ordering Information

Catalog No.	DreamTaq Hot Start DNA Polymerase, 5 U/µL	10X DreamTaq Buffer*
EP1701	200 U	1.25 mL
EP1702	500 U	2 × 1.25 mL
EP1703	2500 U	10 × 1.25 mL
EP1704	4 × 2500 U	40 × 1.25 mL

* includes 20 mM $MgCl_2$

Store at –20°C

www.thermofisher.com

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

DESCRIPTION

Thermo Scientific™ DreamTaq™ Hot Start DNA Polymerase is an enhanced hot start *Taq* DNA polymerase optimized for most PCR applications. It ensures higher sensitivity, specificity, and yields compared to conventional hot start *Taq* DNA polymerase. It is capable of amplifying long amplicons such as 6 kb genomic DNA and 20 kb λ DNA.

DreamTaq Hot Start DNA Polymerase combines *Taq* DNA polymerase and a specific antibody that inhibits the DNA polymerase activity at ambient temperatures, thus preventing the amplification of non-specific products. At polymerization temperatures, the antibody molecule is released, rendering the polymerase fully active.

DreamTaq Hot Start DNA Polymerase uses the same reaction set-up and cycling conditions as conventional *Taq* DNA polymerases, but the antibody-based hot start allows the reactions to be set up at room temperature. Because the enzyme is supplied with the optimized DreamTaq buffer, which includes 20 mM $MgCl_2$, extensive optimization of reaction conditions is not required.

DreamTaq Hot Start DNA Polymerase generates PCR products with 3'-dA overhangs. The enzyme tolerates dUTP and can incorporate modified nucleotides.

FEATURES

- High specificity due to antibody based hot start.
- Robust amplification with minimal optimization.
- High yields of PCR products.
- Higher sensitivity compared to conventional hot start *Taq* DNA polymerases.
- Amplification of long targets up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- Generates 3'-dA overhangs.
- Incorporates dUTP and modified nucleotides.

APPLICATIONS

- Routine PCR amplification of DNA fragments up to 6 kb from genomic DNA and up to 20 kb from viral DNA.
- RT-PCR.
- Genotyping.
- Generation of PCR products for TA cloning.

CONCENTRATION

5 U/µL

DEFINITION OF ACTIVITY UNIT

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 minutes at 74°C.

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10X DREAMTAQ BUFFER

DreamTaq Buffer is a proprietary formulation, which contains KCl and $(NH_4)_2SO_4$ at a ratio optimized for robust performance of DreamTaq Hot Start DNA Polymerase in PCR. DreamTaq Buffer also includes $MgCl_2$ at a concentration of 20 mM.

INHIBITION AND INACTIVATION

- Inhibitors: Ionic detergents (deoxycholate, sarkosyl, and SDS) at concentrations higher than 0.06, 0.02, and 0.01%, respectively.
- Inactivated by phenol/chloroform extraction.

PROTOCOL

To set up parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers, and DreamTaq Hot Start DNA Polymerase. Prepare sufficient master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes, then add template DNA.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. For each 50-µL reaction, add the following components into a thin-walled PCR tube:

10X DreamTaq Buffer*	5 µL
dNTP Mix, 2 mM each (#R0241)	5 µL (0.2 mM of each)
Forward primer	0.1–1.0 µM
Reverse primer	0.1–1.0 µM
Template DNA	10 pg–1 µg
DreamTaq Hot Start DNA Polymerase	1.25 U
Water, nuclease-free (#R0581)	to 50 µL
Total volume	50 µL

*10X DreamTaq Buffer contains 20 mM $MgCl_2$, which is optimal for most applications. If further optimization is required, additional $MgCl_2$ can be added to the master mix. The volume of water should be reduced accordingly.

Volumes of 25 mM $MgCl_2$ (#R0971), required for specific final $MgCl_2$ concentration:

Final concentration of $MgCl_2$	2 mM	2.5 mM	3 mM	4 mM
Volume of 25 mM $MgCl_2$ to be added for 50-µL reaction	0 µL	1 µL	2 µL	4 µL

3. Gently vortex the samples and briefly centrifuge.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Place the reactions in a thermal cycler. 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	30 s	
Extension*	72	1 min	
Final Extension	72	5–15 min	1

* The recommended extension step is 1 minute for PCR products up to 2 kb. For longer products, the extension time should be prolonged by 1 minute/kb.

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. Follow the general recommendations below to lower the risk of contamination.

- 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 use pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Use PCR-certified reagents, including high quality water (e.g., Water, nuclease-free, #R0581).
- Always perform "no template control" (NTC) reactions to check for contamination.

DreamTaq Hot Start DNA Polymerase incorporates dUTP; therefore, you can control carry-over contamination using Uracil-DNA Glycosylase (#EN0361).

GUIDELINES FOR PRIMER DESIGN

Use special design software or follow the general recommendations for PCR primer design as outlined below to design optimal primers:

- Use PCR primers that are 15–30 nucleotides long.
- 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.

(continued on reverse page)

- Avoid self-complementary primer regions, and complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementarity between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3'-end.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

ESTIMATION OF PRIMER MELTING TEMPERATURE

For primers containing less than 25 nucleotides, the approximate 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 to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amount of template DNA for a 50-µL reaction volume is 1 pg–1 ng for both plasmid and phage DNA, and 100 pg–1 µg for genomic DNA. Higher amounts of template increase the risk of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

All routine DNA purification methods are suitable for template preparation; 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, can inhibit DNA polymerases. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol normally removes trace contaminants from DNA samples.

$MgCl_2$ concentration

DreamTaq Hot Start DNA Polymerase is provided with an optimized 10X DreamTaq Buffer, which includes $MgCl_2$ at a concentration of 20 mM. A final $MgCl_2$ concentration of 2 mM is generally ideal for PCR. $MgCl_2$ concentration can be further increased up to 4 mM by the addition of 25 mM $MgCl_2$ (#R0971).

If the DNA samples contain EDTA or other metal chelators, Mg^{2+} ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds 1 Mg^{2+}).

dNTPs

The recommended final concentration of each dNTP is 0.2 mM. In certain PCR applications, higher dNTP concentrations may be necessary. It is essential to have equal concentrations of all four nucleotides (dATP, dCTP, dGTP and dTTP) in the reaction mixture.

To obtain a 0.2 mM concentration of each dNTP in the PCR mixture, refer to the table below.

Volume of PCR mixture	dNTP Mix, 2 mM each (#R0241)	dNTP Mix, 10 mM each (#R0191)	dNTP Mix, 25 mM each (#R1121)
50 µL	5 µL	1 µL	0.4 µL
25 µL	2.5 µL	0.5 µL	0.2 µL
20 µL	2 µL	0.4 µL	0.16 µL

Use 200 µM of each dNTP. dUTP or dITP can be added up to 200 µM. For longer amplicons, a lower dUTP concentration (20–100 µM) may be required for high yields.

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.

CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

DreamTaq Hot Start DNA polymerase is inactive at room temperature during the reaction set up and is activated during the 1–3 minute initial denaturation/enzyme activation step.

It is essential to completely denature the template DNA at the beginning of the PCR run to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 60% or less, an initial 1–3 minute denaturation at 95°C is sufficient. For GC-rich templates this step can be prolonged.

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 minutes. DNA denaturation can also be enhanced by the addition of 5–10% glycerol, 5% DMSO, 1% formamide, or 1–1.5 M betaine. The melting temperature of the primer-template complex decreases significantly in the presence of these reagents. Therefore, the annealing temperature has to be adjusted accordingly.

Note that higher than 10% DMSO or 5% formamide in the reaction mix inhibit DNA polymerases. Therefore, it may be necessary to increase the amount of the enzyme in the reaction if these additives are used.

Primer annealing

The annealing temperature should be equal to 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. When additives that change the melting temperature of the primer-template complex are used (glycerol, DMSO, formamide and betaine), the annealing temperature must also be adjusted.

Extension

The optimal extension temperature for DreamTaq Hot Start DNA Polymerase is 70–75°C. The recommended extension step is 1 minute at 72°C for PCR products up to 2 kb. For longer products, the extension time should be increased by 1 minute/kb. For amplification of templates >6 kb, we recommend reducing the extension temperature to 68°C.

Number of cycles

The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield.

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

Final extension

After the last cycle, we recommend incubating the PCR mixture at 72°C for an additional 5–15 minutes to fill in any possible incomplete reaction products. If the PCR product will be cloned into TA vectors such as the Thermo Scientific™ InsTAclone™ PCR Cloning Kit (#K1213), the final extension step may be prolonged to 15 minutes to ensure the complete 3'-dA tailing of the PCR product. If the PCR product will be used for cloning using Thermo Scientific™ CloneJET™ PCR Cloning Kit (#K1231), the final extension step can be omitted.

TROUBLESHOOTING

For troubleshooting, visit **www.thermofisher.com.**

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable conversion of supercoiled plasmid DNA to a nicked form was observed.

Residual Activity Assay

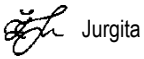
No detectable extension of labeled double stranded oligonucleotide with 5'-overhangs after incubation in the presence of dNTPs.

E. coli DNA Assay

No detectable E.coli DNA was observed.

Functional Assay

Performance in PCR is tested by the amplification of a 594 bp and 7.5 kb fragments of human genomic DNA.

Quality authorized by:  Jurgita Zilinskiene

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.

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

Refer to **www.thermofisher.com/support** for the Safety Data Sheets.

Thermo SCIENTIFIC

PRODUCT INFORMATION

Eco130I (Styl)

#ER0411 2500 U

Lot: ___ **Expiry Date: _**

5'...**C↓C W W G G**...3'

3'...**G G W W C↑C**...5'

Concentration:	10 U/µL
Source:	*Escherichia coli* RFL130
Supplied with:	1 mL of 10X Buffer O
	1 mL of 10X Buffer Tango

Store at -20°C

IBSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer O (for 100% Eco130I digestion)
50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl, 0.1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of Eco130I required to digest 1 µg of lambda DNA in 1 hour at 37°C in 50 µL of recommended reaction buffer.

Dilution
Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl, (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Double Digests
Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.
1X Tango Buffer: 33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Rev.10

Storage Buffer

Eco130I is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer O	2 µL
DNA (0.5-1 µg/µL)	1 µL
Eco130I	0.5-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µL	(~0.1-0.5 µg of DNA)
nuclease-free water	18 µL	
10X Buffer O	2 µL	
Eco130I	1-2 µL	

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

Eco130I is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
0-20	20-50	**100**	50-100	50-100	100

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: never overlaps – no effect.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

A minimum of 0.2 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

C↓CTAGG – BcuI, NheI, XbaI, XmaJI
C↓CATGG – AflIII, PscI, FatI, PagI, BtgI, NcoI.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
10	0	1	0	0	0	0

For **CERTIFICATE OF ANALYSIS** *see* back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Eco130I (10 u/µg lambda DNA x 16 hours).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of Eco130I for 4 hours.

Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

Quality authorized by:  Jurgita Zilinskiene

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.

Thermo SCIENTIFIC

PRODUCT INFORMATION

MnlI

#ER1072 1500 U

Lot: ___ **Expiry Date: _**

5'...**C C T C (N)$_7$**↓...3'
3'...**G G A G (N)$_6$**↑...5'

Concentration: 10 U/µL
Source: *E.coli* that carries the cloned *mnlIR* gene from *Moraxella nonliquefaciens*
Supplied with: 1 mL of 10X Buffer G
1 mL of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Buffer G (for 100% MnlI digestion)
10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of MnlI required to digest 1 µg of lambda DNA in 1 hour at 37°C in 50 µL of recommended reaction buffer.

Dilution
Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C) 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Double Digests
Thermo Scientific Tango Buffer is provided to simplify buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.
1X Tango Buffer: 33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Storage Buffer

MnlI is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer G	2 µL
DNA (0.5-1 µg/µL)	1 µL
MnlI	0.5-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µL	(~0.1-0.5 µg of DNA)
nuclease-free water	18 µL	
10X Buffer G	2 µL	
MnlI	1-2 µL	

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

MnlI is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
50-100	**100**	20-50	20-50	20-50	20-50

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – no effect.
EcoKI: never overlaps – no effect.
EcoBI: may overlap – blocked.

Stability during Prolonged Incubation

A minimum of 0.5 units of the enzyme is required for complete digestion of 1 µg of DNA in 16 hours at 37°C.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
262	34	26	14	13	12	61

Note

- MnlI produces DNA fragments that have a single-base 3'-extension which are more difficult to ligate than blunt-ended fragments.
- MnlI may remain associated with the cleaved DNA. This may cause DNA band shifting during electrophoresis. To avoid atypical DNA band patterns, use the 6X DNA Loading Dye&SDS Solution (#R1151) for sample preparation or heat the digested DNA in the presence of SDS prior to electrophoresis.

For **CERTIFICATE OF ANALYSIS** *see* back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with MnlI (10 U/µg lambda DNA x 16 hours).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of MnlI for 4 hours.

Quality authorized by:  Jurgita Zilinskiene

Thermo SCIENTIFIC

PRODUCT INFORMATION

Eco88I (AvaI)

#ER0381 1000 U

Lot: ___ **Expiry Date: _**

5'...**C↓Y C G R G**...3'
3'...**G R G C Y↑C**...5'

Concentration: 10 U/µL
Source: *E.coli* that carries the cloned *eco88IR* gene from *E.coli* RFL88
Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% Eco88I digestion)
33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of Eco88I required to digest 1 µg of lambda DNA in 1 hour at 37°C in 50 µL of recommended reaction buffer.

Dilution
Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Double Digests
Tango™ Buffer provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer
Eco88I is supplied in: 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Rev.11

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer Tango	2 µL
DNA (0.5-1 µg/µL)	1 µL
Eco88I	0.5-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µL	(~0.1-0.5 µg of DNA)
nuclease-free water	18 µL	
10X Buffer Tango	2 µL	
Eco88I	1-2 µL	

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

Eco88I is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
100	50-100	0-20	0-20	**100**	20-50

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – cleavage impaired.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Stability during Prolonged Incubation

A minimum of 0.2 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

Digestion of Agarose-embedded DNA

A minimum of 5 units of the enzyme is required for complete digestion of 1 µg of agarose-embedded lambda DNA in 16 hours.

Compatible Ends

C↓CCGGG - BshTI, BsaWI, Cfr9I, Cfr10I, Kpn2I, MreI, NgoMIV, SgrAI
C↓TCGAG - SalI, SgrDI, SmoI, XhoI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
8	1	1	1	1	1	2

For **CERTIFICATE OF ANALYSIS** *see* back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is after a 160-fold overdigestion with Eco88I (10 U/µg lambda DNA × 16 hours).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of Eco88I for 4 hours.

Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

Quality authorized by:  Jurgita Zilinskiene

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.

Thermo SCIENTIFIC

PRODUCT INFORMATION

HincII (HindII)

#ER0491 500 U

Lot: ___ **Expiry Date: _**

5'...**G T Y↓R A C**...3'
3'...**C A R↑Y T G**...5'

Concentration: 10 U/µL
Supplied with: 1 mL of 10X Buffer Tango

Store at -20°C

BSA included

www.thermoscientific.com/onebio

RECOMMENDATIONS

1X Thermo Scientific Tango Buffer (for 100% HincII digestion)
33 mM Tris-acetate (pH 7.9), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/mL BSA.

Incubation temperature
37°C.

Unit Definition
One unit is defined as the amount of HincII required to digest 1 µg of lambda DNA in 1 hour at 37°C in 50 µL of recommended reaction buffer.

Dilution
Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/mL BSA and 50% glycerol.

Double Digests
Tango™ Buffer provided simplifies buffer selection for double digests. 98% of Thermo Scientific restriction enzymes are active in a 1X or 2X concentration of Tango Buffer. Please go to www.thermoscientific.com/doubledigest to choose the best buffer for your experiments.

Storage Buffer
HincII is supplied in: 10 mM Tris-HCl (pH 7.5 at 25°C), 200 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5 mg/mL BSA and 50% glycerol.

Rev.10

Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µL
10X Buffer Tango	2 µL
DNA (0.5-1 µg/µL)	1 µL
HincII	0.5-2 µL

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

The digestion reaction may be scaled either up or down.

Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µL	(~0.1-0.5 µg of DNA)
nuclease-free water	18 µL	
10X Buffer Tango	2 µL	
HincII	1-2 µL	

- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

Thermal Inactivation

HincII is inactivated by incubation at 65°C for 20 min.

ENZYME PROPERTIES

Enzyme Activity in Thermo Scientific REase Buffers, %

B	G	O	R	Tango	2X Tango
50-100	50-100	20-50	50-100	**100**	50-100

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – cleavage impaired.
EcoKI: may overlap – blocked.
EcoBI: may overlap – blocked.

Stability during Prolonged Incubation

A minimum of 0.1 units of the enzyme is required for complete digestion of 1 µg of lambda DNA in 16 hours at 37°C.

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
35	13	2	1	1	1	1

For **CERTIFICATE OF ANALYSIS** *see* back page

CERTIFICATE OF ANALYSIS

Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with HincII (10 U/µg lambda DNA × 16 hours).

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of HincII for 4 hours.

Quality authorized by:  Jurgita Zilinskiene

PRODUCT INFORMATION

Thermo Scientific GeneRuler 50 bp DNA Ladder

Pub. No. MAN0013016
Rev. Date 12 February 2018 (Rev. C.00)

Components	#SM0371	#SM0372
GeneRuler 50 bp DNA Ladder, 0.5 µg/µL	50 µg (for 100 applications)	250 (5 x 50) µg (for 500 applications)
6X TriTrack DNA Loading Dye	1 mL	2 × 1 mL

Store at -25°C to -15°C

www.thermofisher.com

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

Description

Thermo Scientific™ GeneRuler™ 50 bp DNA Ladder 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 dissolved in TE buffer.

Storage Buffer

10 mM Tris-HCl (pH 7.6), 1 mM EDTA.

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

Loading mixture for the 5 mm gel lane*:

Components	Gels	
	Agarose	**Polyacrylamide**
DNA ladder (0.5-1 µg)	1-2 µL	1-2 µL
6X TriTrack DNA Loading Dye	1 µL	0.5 µL
Deionized water	4-3 µL	1.5-0.5 µL
	6 µL	3 µL

Step 1: Mix gently

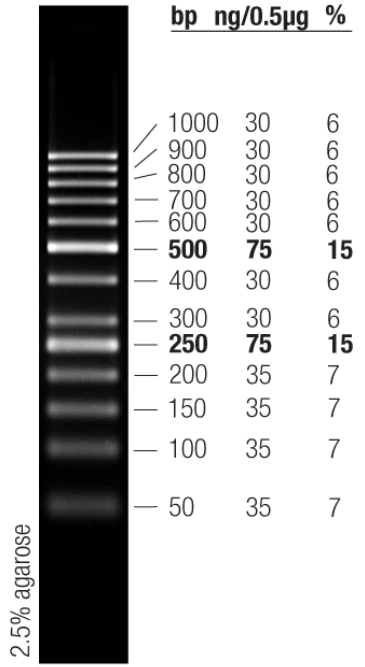
Step 2: Load on the gel

*For gels with other lane widths, the components of the mixture should be scaled either up or down. Use 0.2-0.4 µL (0.1-0.2 µg) of DNA ladder per 1 mm of lane.

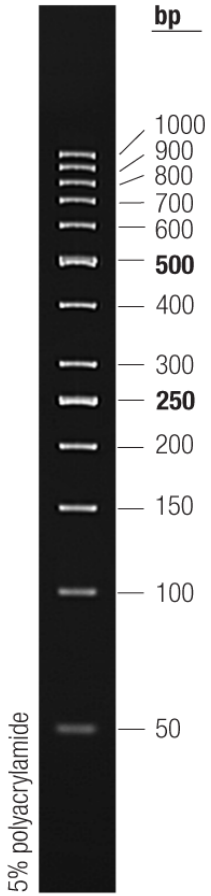
Recommendations

- 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



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.

pUC19 DNA/MspI (HpaII) Marker, 23

Catalog Number SM0221, SM0222

Pub. No. MAN0012992 **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
SM0221	pUC19 DNA/MspI (HpaII) Marker, 23	50 µg (for 100 applications), 0.5 µg/µL	-25 °C to -15 °C
	6X DNA Loading Dye	1 mL	
SM0222	pUC19 DNA/MspI (HpaII) Marker, 23	250 (5 x 50) µg (for 500 applications), 0.5 µg/µL	
	6X DNA Loading Dye	2 x 1 mL	

Description

pUC19 DNA was completely digested with MspI, purified and dissolved in a storage buffer.
The DNA Marker contains the following 13 discrete fragments (in base pairs): 501, 489, 404, 331, 242, 190, 147, 111, 110, 67, 34, 34, 26.

Storage Buffer

10 mM Tris-HCl (pH 7.6), 1 mM EDTA.

6X DNA Loading Dye

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

Protocol for Loading

Loading mixture for the 5 mm gel lane*:

Components	Gels	
	Agarose	**Polyacrylamide**
DNA ladder (0.5-1 µg)	1-2 µL	1-2 µL
6X DNA Loading Dye	1 µL	0.5 µL
Deionized water	4-3 µL	1.5-0.5 µL
	6 µL	3 µL

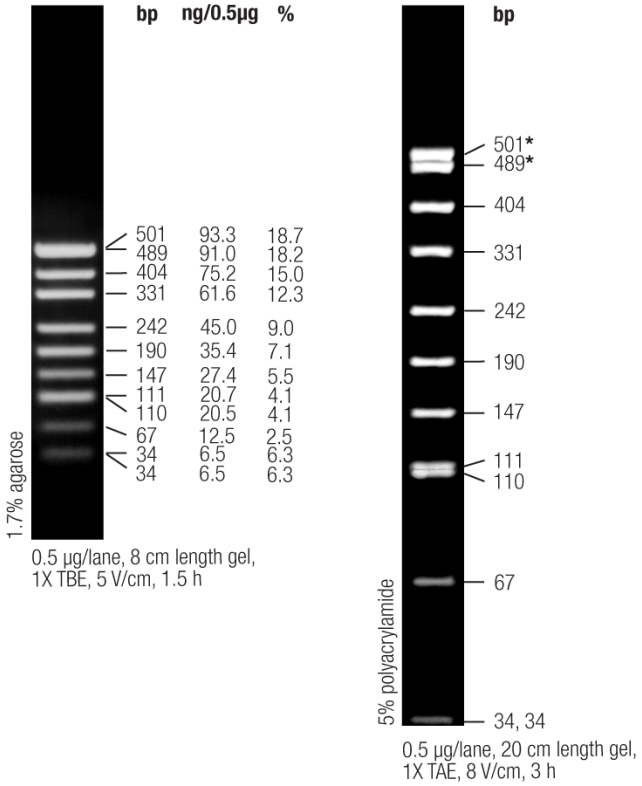
Step 1: Mix gently
Step 2: Load on the gel

*For gels with other lane widths, the components of the mixture should be scaled either up or down. Use 0.2-0.4 µL (0.1-0.2 µg) of DNA ladder per 1 mm of lane.

Recommendations

- Do not heat before loading
- Dilute your DNA sample with the 6X DNA Loading Dye (#R0611, supplied with the ladder): mix 1 volume of the dye solution with 5 volumes of the DNA sample;
- 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.

pUC19 DNA/MspI (HpaII) Marker, 23



*The 501 and 489 bp bands migrate anomalously (1, 2, 3).
26 bp fragment is not visible and comprises 1.0 %

References

1. Stellwagen, N.C., Anomalous electrophoresis of deoxyribonucleic acid restriction fragments on polyacrylamide gels, Biochemistry, 22, 6186-6193, 1983.
2. Lane, D., et al., Use of gel ratardation to analyze protein – nucleic acid interactions, Microbiological Reviews, 56, 509-528, 1992.
3. Stellwagen, N.C., Conformational isomers of curved DNA molecules can be observed by polyacrylamide gel electrophoresis, Electrophoresis, 21, 2327-2334, 2000.

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**.

SYBR™ Safe DNA Gel Stain

Catalog Number S33100, S33101, S33102, S33103, S33110, S33111, S33112

Pub. No. MAN0002338 (MP33100) **Rev.** A.0

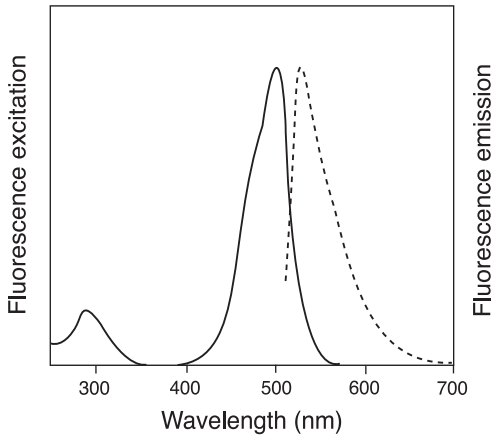
Table 1 Contents and storage

Material	Cat. No.	Amount	Concentration	Storage	Stability
SYBR™ Safe in TBE buffer	S33100	1 L*	0.5X, 45 mM Tris-borate, 1 mM EDTA, pH ~8.3	• 2–25°C • Protect from light	~6 months, when stored as directed
	S33101	4 L†			
SYBR™ Safe in TAE buffer	S33111	1 L*	1X, 40 mM Tris-acetate, 1 mM EDTA, pH ~8.3		
	S33112	4 L†			
SYBR™ Safe in DMSO	S33102	400 µL	10,000X		
	S33103	50 µL			
SYBR™ Safe DNA Gel Stain Starter Kit	S33110	1 L of SYBR™ Safe gel stain, and one SYBR™ Safe photographic filter	0.5X TBE		
Number of labelings: * Provides sufficient material to stain ~20 minigels. **†** Provides sufficient material to stain ~80 minigels. 4 L unit size is packaged in a cube-shaped container with a removable spigot for easy dispensing and storage.					
Approximate fluorescence excitation and emission maxima: 280, 502/530 nm, bound to DNA					

Introduction

SYBR™ Safe DNA gel stain has been specifically developed for reduced mutagenicity, making it safer than ethidium bromide for staining DNA in agarose or acrylamide gels. SYBR™ Safe stain comes either as a concentrate or as a ready-to-use solution that can be used like an ethidium bromide solution, and the detection sensitivity with SYBR™ Safe stain is comparable to that obtained with ethidium bromide. DNA bands stained with SYBR™ Safe DNA gel stain can be detected using a standard UV transilluminator, a visible-light transilluminator, or a laser-based scanner. The stain is also suitable for staining RNA in gels. Bound to nucleic acids, SYBR™ Safe stain has fluorescence excitation maxima at 280 and 502 nm, and an emission maximum at 530 nm (Figure 1, page 2).

Figure 1 Normalized fluorescence excitation and emission spectra of SYBR™ Safe DNA gel stain, determined in the presence of DNA



Before starting

Storage, handling, and disposal

You may store the SYBR™ Safe DNA gel stain at any temperature between 2°C to 25°C. SYBR™ Safe in DMSO freezes at low temperatures; therefore, the product must be completely thawed and mixed before using. Repeated freeze-thawing has minimal impact on product performance.

SYBR™ Safe DNA gel stain showed no or very low mutagenic activity when tested by an independent, licensed testing laboratory, and this stain is not classified as hazardous waste under U.S. Federal regulations. The safety testing included 3 well-established mammalian cell–based tests (Table 2, page 3), a battery of well-established Ames-test bacterial strains (Figure 2, below), and extensive testing for environmental safety (Tables 3 and 4, page 3). Use care when using this reagent and dispose of the stain in compliance with all pertaining local regulations.

Figure 2 Summary of Ames test results for mutagenicity. Samples were pre-treated with a mammalian S9 fraction and then tested using the indicated Ames test strain. With strains TA97a, TA98, TA100, and TA102, a result of less than two-fold above background suggests that the compound is nonmutagenic; whereas, a result of greater than this value suggests that the compound is mutagenic. With strains TA1535, TA1537, and TA1538, a result of less than three-fold above background suggests that the compound is nonmutagenic; whereas, a result of greater than this value suggests that the compound is mutagenic. All tests were performed by Covance Laboratories, Inc., Vienna, VA, an independent testing laboratory.

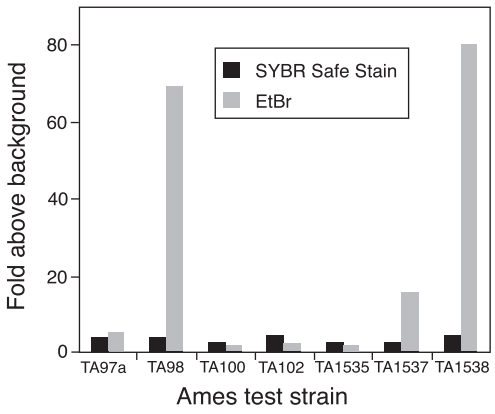


Table 2 Summary of mammalian cell–based tests for DNA genotoxicity

Test*	Cell type	Test result with S9 activation†	Test result without S9 activation†
Transformation test [1]	Syrian hamster embryo (SHE) cells	Not applicable	Negative
Chromosomal aberration test [2]	Cultured human peripheral blood lymphocytes	Negative	Negative
Forward-mutation test [3,4]	L5178Y TK mouse lymphoma cells	Negative	Negative

***** All tests were performed by Covance Laboratories, Inc., Vienna, VA, an independent testing laboratory. **†** S9, a mammalian extract obtained from Aroclor™ 1254-induced rat liver.

1. Fundamental and Molecular Mechanisms of Mutagenesis 356:1 (1996); **2.** Evans, H.J., in *Chemical Mutagens, Principles and Methods for their Detection Vol 4,* A. Hollaender, Ed., Kluwer Academic/ Plenum Publishers (1976) pp. 1–29; **3.** Mutation Res 72, 447 (1980); **4.** Mutation Res 59, 61 (1979).

Table 3 Summary of environmental safety test results

Test*	Method	Results
Aquatic toxicity	Fathead minnow CA Title 22 acute screening	Not classified as hazardous or toxic to aquatic life
Ignitability	EPA 1010	Not ignitable (>100°C)
Corrosivity	EPA 150.1	Not corrosive (pH = 8.25)
Corrosivity (by Corrositex)	DOT-E 10904	Category 2 noncorrosive
Reactivity	EPA 9010B/9030A	No reactivity detected

***** All tests were independently confirmed by AMEC Earth and Environmental San Diego Bioassay Laboratory, San Diego, CA.

Table 4 Summary of pollutant discharge test results

Test*	SYBR™ Safe Stain†	0.5X TBE
pH (150.1)	8.45	8.48
Total cyanide (335.2)	None detected	None detected
Chemical oxygen demand (COD; 410.1)	7020	6840
Ammonia as nitrogen (350.1)	253	248
Total organic carbon (415.1)	2480	2360
Total phenolics (420.1)	None detected	None detected
Organochlorine pesticides and PCBs (608M)	None detected	None detected
Semi-volatile organic compounds (625)	None detected	None detected
Volatile organic compounds (624)	None detected	None detected
Metals (6010B, 7060A, 7421, 7470A, 7740, 7841)	None detected	None detected

***** Code of Federal Regulations Title 40, Part 136; **†** 1X SYBR™ Safe stain (Lot X40023) in 0.5X TBE.

Staining nucleic acids after electrophoresis

1.1 Soak the gel in SYBR™ Safe stain. If using SYBR™ Safe gel stain concentrate, dilute 10,000X in TAE or TBE buffer (as appropriate) prior to use. Place the gel in a plastic container, such as a pipet-tip box lid or a household food-storage container. Do not use a glass container, because the dye in the staining solution may adsorb to the walls of the container, resulting in poor gel staining. Add sufficient SYBR™ Safe DNA gel stain to cover the gel. A 50 mL volume is sufficient for staining most standard minigels. To stain larger gels, increase the volume of staining solution in proportion to the increased gel volume, and ensure that the gel is fully immersed during staining.

1.2 Incubate for 30 minutes. Cover the gel and the staining solution with aluminum foil or place them in the dark to protect from light. Continuously agitate the gel on an orbital shaker at 50 rpm. No destaining is required.

Precasting SYBR™ Safe Stain in agarose gels

2.1 Prepare the agarose gel directly in SYBR™ Safe DNA gel stain. SYBR™ Safe stain is provided in buffer; simply substitute SYBR™ Safe stain for the buffer when preparing the molten agarose. If using the 10,000X SYBR™ Safe stain concentrate, dilute the concentrated stain 1:10,000 in agarose gel buffer (e.g., 1X TBE or 1X TAE) and add the buffer plus stain mixture to the powdered agarose. For example if you run TBE gels and require 30 mL of molten agarose for your tray, add 3 µL of 10,000X SYBR™ Safe stain concentrate to 30 mL of 1X TBE, mix well, and add to the powdered agarose.

Note: You can heat the agarose/SYBR™ Safe stain mixture in the microwave. As with precasting gels with ethidium bromide, the mobility of nucleic acid fragments in the gel may be somewhat slower when run in these gels compared to their mobility in the gel without stain.

2.2 Run the gel. Use a running buffer appropriate to the SYBR™ Safe gel stain formulation. No post-staining or destaining is needed.

Viewing and photographing the gel

You can view stained gels using a standard 300 nm transilluminator, a 254 nm epi- or transilluminator, or a blue-light transilluminator such as the Safe Imager™ 2.0 Blue-Light transilluminator (Cat. no. G6600). DNA stained with SYBR™ Safe stain can also be visualized and analyzed using imaging systems equipped with an excitation source in the UV range or between 470–530 nm. Refer to Table 5 on page 5 to determine the optimal filter sets to use, or contact the instrument manufacturer for advice.

Note: If bands from the SYBR™ Safe stained gel are to be excised and used in a ligation reaction, we recommend that the gel is illuminated using blue-light source (i.e., Safe Imager™ 2.0 Blue-Light transilluminator) and not a UV light source. In some instances, UV light sources in combination with SYBR™ Safe stain can lead to reduced cloning efficiencies.

You can photograph stained gels using Polaroid™ 667 black-and-white print film and SYBR™ Safe photographic filter (Cat. no. S37100). SYPRO™ photographic filter (Cat. no. S6656) or a Kodak™ Wratten #9 filter also work well. Using this film and one of these filters, SYBR™ Safe DNA gel stain provides the same detection sensitivity as ethidium bromide and an appropriate filter. Do not use a standard ethidium bromide photographic filter with SYBR™ Safe DNA gel stain. Gels stained with SYBR™ Safe stain can also be imaged using a CCD camera or a laser-based scanner.

Table 5 Filter selection guide for use with SYBR™ Safe stain

Instrument (Manufacturer)	Excitation source	Emission filter
AlphaImager (Alpha Innotech)	302 nm	SYB-500
AlphaImager HP (Alpha Innotech)	302 nm	SYB-500
AlphaDigiDoc RT (Alpha Innotech)	UV transilluminator	
Shroud, Camera Stand (Alpha Innotech)	UV transilluminator	SYB-100
DE500 or DE400 light cabinet 2.17" diameter (Alpha Innotech)	UV transilluminator	SYB-500
DE500 or DE400 light cabinet 2" diameter (Alpha Innotech)	UV transilluminator	SYB-400
VersaDoc Imaging Systems (Bio-Rad)	Broadband UV	520LP
Molecular Imager FX Systems (Bio-Rad)	488 nm	530 nm BP
Gel Doc Systems (Bio-Rad)	302 nm	520DF30 (#170-8074)
Typhoon 9400/9410 (GE Healthcare)	488 nm	520 BP 40
Typhoon 9200/9210/8600/8610 (GE Healthcare)	488 nm	526 SP
FluorImager (GE Healthcare)	488 nm	530 DF 30
Storm (GE Healthcare)	Blue (fluorescence mode)	
VDS-CL (GE Healthcare)	Transmission	UV Low
Ultracam/Gel Imager (Ultra-Lum)	UV	Yellow Filter (#990-0804-07)
Omega Systems (Ultra-Lum)	UV	520 nm
Polaroid Camera (Polaroid)	UV	SYBR™ Safe Photographic Filter (S37100)
FOTO/Analyst Express/Investigator/Plus/ Luminary (FOTODYNE)	UV	Fluorescent Green (#60-2034)
FOTO/Analyst Minivisionary (FOTODYNE)	UV	Fluorescent Green (#62-4289)
FOTO/Analyst Apprentice (FOTODYNE)	UV	Fluorescent Green (#62-2535)
FOTO/Analyst Luminary (FOTODYNE)	UV	Fluorescent Green (#60-2056)
FCR-10 (Polaroid)	UV	#3-4218
FUJI FLA-3000 (FUJI Film)	473 nm	520LP
BioDocIt/AC1/EC3/BioSpectrum (UVP)	302 nm	SYBR™ Green (#38-0219-01) or SYBR™ Gold (#38-0221-01)
Gel Logic (Kodak)	UV	535 nm WB50
Syngene Instruments (Syngene)	UV	500–600 nm Shortpass filter

Product list Current prices may be obtained at www.thermofisher.com or from our Customer Service Department.

Cat no.	Product name	Unit size
G6600	Safe Imager™ 2.0 Blue-Light Transilluminator	each
S33100	SYBR™ Safe DNA gel stain in 0.5X TBE	1 L
S33101	SYBR™ Safe DNA gel stain in 0.5X TBE	4 L
S33102	SYBR™ Safe DNA gel stain *10,000X concentrate in DMSO*	400 µL
S33103	SYBR™ Safe DNA gel stain *10,000X concentrate in DMSO* *sample size*	50 µL
S33110	SYBR™ Safe DNA Gel Stain Starter Kit *with 1 L of SYBR™ Safe DNA gel stain in 0.5X TBE (S33100) and one photographic filter (S37100)*	1 kit
S33111	SYBR™ Safe DNA gel stain in 1X TAE	1 L
S33112	SYBR™ Safe DNA gel stain in 1X TAE	4 L
S37100	SYBR™ Safe photographic filter	each
G5218-01	E-Gel™ 1.2% with SYBR™ Safe	18 gels
G6206-01	E-Gel™ 1.2% with SYBR™ Safe Starter Kit	1 kit
G5218-02	E-Gel™ 2.0% with SYBR™ Safe	18 gels
G6206-02	E-Gel™ 2.0% with SYBR™ Safe Starter Kit	1 kit

Purchaser notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining support
For the latest services and support information for all locations, go to **thermofisher.com/support**.

At the website, you can:
- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**thermofisher.com/support**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS
Safety Data Sheets (SDSs) are available at **thermofisher.com/support**.

Certificate of Analysis
The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **thermofisher.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the product packaging (tube, pouch, or box).

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TaqMan QSY probes

New quencher available for your qPCR probes

Applied Biosystems™ TaqMan™ QSY™ probes incorporate a proprietary nonfluorescent 3´ QSY quencher to provide maximal PCR performance in a multiplex format (Figure 1). Experience the sensitivity and specificity you know and expect from TaqMan™ Assays, with another great option for your real-time PCR assay designs.

Figure 1. QSY probe. The newly developed QSY quencher can be used in multiplex qPCR with FAM™, VIC™, ABY™, and JUN™ reporter dyes. The QSY quencher is nonfluorescent, leading to less background and improved quenching efficiency.

QSY probes are comparable to BHQ probes

Your current Black Hole Quencher™ (BHQ™) probe designs can easily be converted to QSY probes. Identical sequence designs can be used with similar performance using FAM dye (Figure 2) and improved performance using our ABY™ dye (Figure 3).

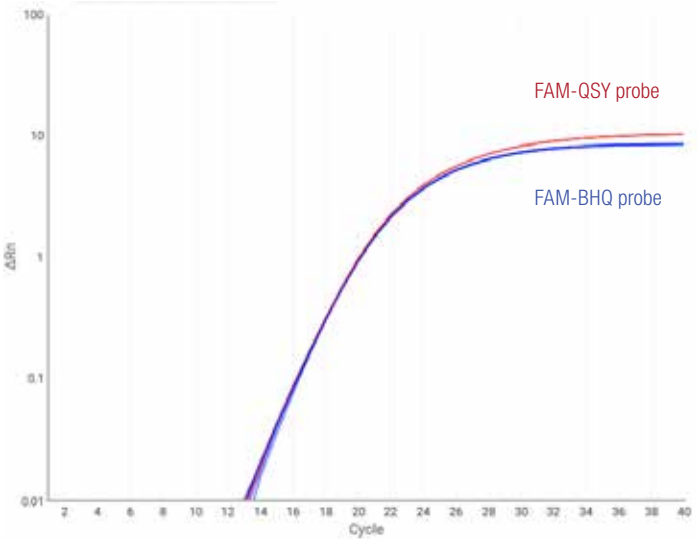

Figure 2. QSY probes have performance similar to that of BHQ probes. A FAM-QSY probe and a FAM-BHQ probe with identical oligonucleotide sequences and master mixes have similar C_t values.

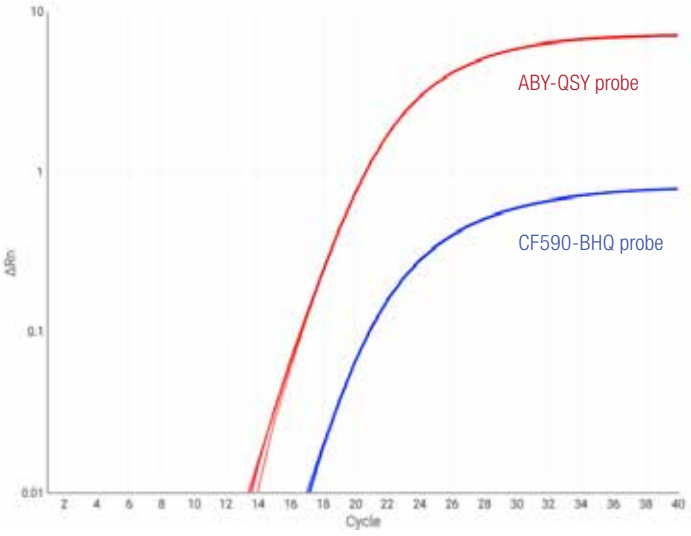

Figure 3. Improved performance in multiplex qPCR. In this multiplex experiment, the ABY-QSY probe shows a significantly lower C_t than the CF590-BHQ probe with an identical oligonucleotide sequence and master mix.

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Four dye options optimized with our instruments for better sensitivity

TaqMan QSY probes can be ordered with FAM, VIC, and our proprietary ABY and JUN dyes, allowing amplification of up to 4 targets in a single reaction. All 4 dyes are optimized for the filter sets on Applied Biosystems™ real-time PCR instruments (Figure 4) and work together with minimal spectral overlap for optimal performance.

Performance without compromise

Multiplexing with TaqMan QSY probes enables cost savings and preservation of limited samples, and also yields comparable results between reactions performed in individual tubes and in 4-plex reactions, for a gene quantification experiment (Figure 5).

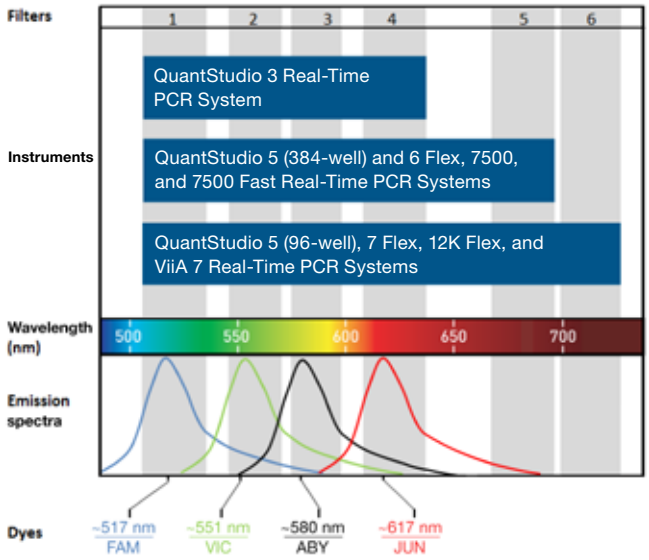

Figure 4. Fluorescence emission wavelengths used for multiplex real-time PCR. Emission spectra for FAM, VIC, ABY, and JUN dyes are shown in relation to regions of the spectrum detected by six filters available on Applied Biosystems real-time PCR instruments.

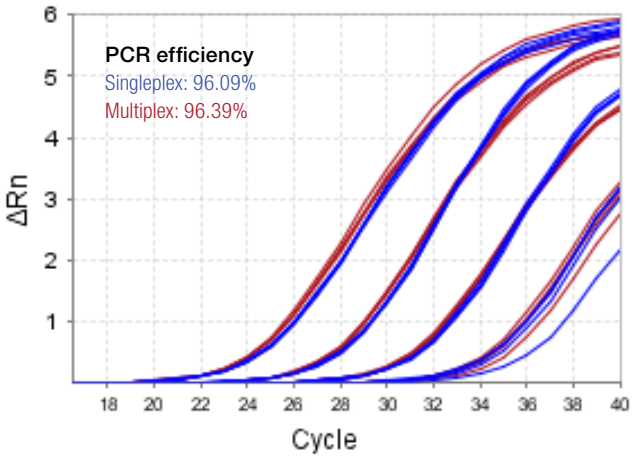

Figure 5. Comparable results for singleplex and multiplex assays. The amplification plot shows linear portions of the curves for 4 EGFR assays amplified in singleplex (blue) and 4-plex reactions (red) in a dilution series from 20,000 pg to 2 pg of reference colon cDNA per 10 µL reaction. PCR efficiencies are 96.09% for EGFR singleplex and 96.39% for EGFR 4-plex reactions.

Ordering information

Product	Quantity	Cat. No.
TaqMan QSY Probe	6,000 pmol	4482777
TaqMan QSY Probe	20,000 pmol	4482778
TaqMan QSY Probe	50,000 pmol	4482779

Product	Quantity	Cat. No.
TaqMan Multiplex Master Mix (2X)	5 mL	4461882
TaqPath 1-Step Multiplex Master Mix (4X)	5 x 1 mL	A28526
TaqPath 1-Step Multiplex Master Mix, No ROX (4X)	5 x 1 mL	A28522
Spectral Calibration Plate for Multiplex qPCR	1 plate	Various

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

ThermoFisher SCIENTIFIC

3500 Dx and 3500xL Dx Genetic Analyzers

Flexibility for clinical research applications with the Research Use Only (RUO) Mode

Key features supporting RUO applications

- Advanced thermal system design improves temperature control for demanding DNA fragment analysis applications
- Superior signal uniformity in fragment sizing applications from instrument to instrument, run to run, and capillary to capillary
- Exceptional application flexibility—one array and one polymer are used for most applications
- No consumable-based instrument hard stops, giving you control of how much and how long consumables are utilized

3500 Dx Series system specifications for RUO applications

Applied Biosystems™ 3500 Dx and 3500xL Dx Genetic Analyzers can perform a number of sequencing and high-resolution fragment sizing applications for research use, including but not limited to STR/microsatellite analysis, loss of heterozygosity (LOH), and SNP confirmation and screening, as well as *de novo* sequencing and resequencing (mutation profiling).

A full range of applications may be run using a single polymer (Applied Biosystems™ POP-7™ Polymer) and the 50 cm capillary array. For even greater application versatility, Applied Biosystems™ POP-4™ and POP-6™ Polymers and the 36 cm capillary array are also available.

Secondary analysis software for RUO applications

- Applied Biosystems™ Sequencing Analysis Software with KB™ Basecaller for sequence basecalling, editing, re-basecalling, reporting, and printing
- Applied Biosystems™ Variant Reporter™ Software for mutation detection, SNP discovery, comparative sequencing, resequencing, validation, and sequence confirmation
- Applied Biosystems™ SeqScape™ Software for resequencing applications with library identification
- Applied Biosystems™ GeneMapper™ Software for microsatellite, LOH, SNP, and t-RFLP analyses
- Applied Biosystems™ GeneMapper™ *ID-X* Software for analysis of human identification data using AmpFℓSTR™ kits
- Applied Biosystems™ MicroSEQ™ ID Analysis Software for microbial sequence typing using MicroSEQ™ kits

Product	Cat. No.
Anode Buffer Container, CE-IVD	4393925
Capillary Array, 8-capillary, 36 cm, CE-IVD	4404682
Capillary Array, 24-capillary, 36 cm, CE-IVD	4404686
Capillary Array, 8-capillary, 50 cm, CE-IVD	4404684
Capillary Array, 24-capillary, 50 cm, CE-IVD	4404688
Cathode Buffer Container, CE-IVD	4408258
Conditioning Reagent, CE-IVD	4409543
POP-6 Polymer (960 samples), CE-IVD	4393711
POP-6 Polymer (384 samples), CE-IVD	4393716
POP-7 Polymer (960 samples), CE-IVD	4393713
POP-7 Polymer (384 samples), CE-IVD	4393709
POP-4 Polymer (960 samples)	4393710
POP-4 Polymer (384 samples)	4393715
Hi-Di Formamide (4 x 5 mL), CE-IVD	4404307
BigDye Terminator Sequencing Standard v1.1, CE-IVD	4462113
BigDye Terminator Sequencing Standard v3.1, CE-IVD	4404310
BigDye Terminator v3.1 Matrix Standards Kit, 3500/3500xL	4336974
BigDye Terminator v1.1 Matrix Standards Kit, 31xx and 3500	4336824
DS-33 Matrix Standard Kit (5-dye), CE-IVD	A25775
DS-33 GeneScan Install Standards, CE-IVD	A25793
DS-02 Matrix Standard Kit (5-dye, E5 dye set)	4323014
DS-32 Matrix Standard Kit (4-dye)	4345831
DS-36 Matrix Standard Kit (6-dye)	4425042
GeneScan 120 LIZ Size Standard	4322362
GeneScan 500 ROX Size Standard	401734
GeneScan 600 LIZ Size Standard v2.0, CE-IVD	A25794
GeneScan 1200 LIZ Size Standard	4379950

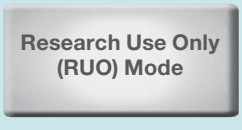

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Table 1. Sequencing modules for RUO applications.

Run modules	Throughput[1]			Configuration		Performance		
	Average run time (minutes)	Average throughput, 3500xL Dx (samples/day)	Average throughput, 3500 Dx (samples/day)	Array separation distance (cm)	Polymer type	Resolution range in ≥90% of samples	Bases collected in 90% of samples	QV20 CRL[2] in ≥90% of samples
ShortReadSeq50_POP7[3]	≤30	≥1,104	≥368	50	POP-7	40–400	≥325	≥300
RapidSeq50_POP7	≤40	≥840	≥280	50	POP-7	40–550	≥600	≥500
FastSeq50_POP7	≤65	≥504	≥168	50	POP-7	40–600	≥750	≥700
StdSeq50_POP7	≤125	≥264	≥88	50	POP-7	40–700	≥1,000	≥850
BDxShortReadSeq50_POP7	≤30	≥1,104	≥368	50	POP-7	40–400	≥325	≥300
BDxRapidSeq50_POP7	≤40	≥840	≥280	50	POP-7	40–550	≥600	≥500
BDxFastSeq50_POP7	≤65	≥504	≥168	50	POP-7	40–600	≥750	≥700
BDxStdSeq50__POP7	≤125	≥264	≥88	50	POP-7	40–700	≥1,000	≥850
RapidSeq50_POP6	≤65	≥504	≥168	50	POP-6	20–500	≥450	≥450
FastSeq50_POP6	≤90	≥368	≥122	50	POP-6	20–550	≥600	≥600
StdSeq50_POP6	≤135	≥240	≥80	50	POP-6	20–600	≥700	≥600
BDxRapidSeq50_POP6	≤65	≥504	≥168	50	POP-6	20–500	≥450	≥450
BDxFastSeq50_POP6	≤90	≥368	≥122	50	POP-6	20–550	≥600	≥600
BDxStdSeq50_POP6	≤140	≥240	≥80	50	POP-6	20–600	≥700	≥600
MicroSEQ ID 50_POP6	≤135	≥240	≥80	50	POP-6	20–600	≥700	≥600
FastMicroSEQ ID 50_POP6	≤105	≥312	≥104	50	POP-6	20–500	≥450	≥425

The specifications are reported using the BigDye Terminator v3.1 Sequencing Standard. BDx classified run modules are optimized with the 3500 Dx Series systems to obtain more usable data when sequencing reactions are purified using the BigDye XTerminator Purification Kit.

1. Throughput (samples/day) is determined by the total number of samples that can be run in 23 hours (allows time for sample preparation, instrument maintenance, and warm-up).
2. QV20 CRL is defined as the longest uninterrupted segment of bases with an average of QV ≥20, calculated over a sliding window of 21 base pairs.
3. The fast ShortReadSeq module collects 300 bp in 30 minutes for operations requiring short verification of sequence content (e.g., clone QC verification).

Table 2. Fragment analysis modules for RUO applications.

Module name	Throughput[1]			Configuration		Performance							
						General		Sizing precision[3] of 100% of alleles in ≥90% of samples			Multirun sizing[4] of 100% of alleles in ≥90% of samples		
	Average run time (minutes)	Average throughput, 3500xL Dx (samples/day)	Average throughput, 3500 Dx (samples/day)	Array length (cm)	Polymer type	Resolution range[2] in ≥90% of samples	Largest fragment collected in ≥90% of samples	50–400 bp	401–600 bp	601–1,200 bp	50–400 bp	401–600 bp	601–1,200 bp
FragmentAnalysis36_POP7	≤30	≥1,104	≥368	36	POP-7	60–400	>420	<0.15	NA	NA	<1 bp	NA	NA
Fragment Analysis36_POP4	≤35	≥936	≥312	36	POP-4	60–400	>420	<0.15	NA	NA	<1 bp	NA	NA
FragAnalysis50_POP7	40	≥840	≥280	50	POP-7	40–520	≥600	<0.15	<0.30	NA	<1 bp	<2 bp	NA
FragAnalysis50_POP6	100	≥336	≥112	50	POP-6	20–550	≥600	<0.15	<0.30	NA	<1 bp	<2 bp	NA
LongFragAnalysis50_POP7	125	≥264	≥88	50	POP-7	40–700	≥1,200	<0.15	<0.30	<0.45	<1 bp	<2 bp	<3 bp
HID36_POP4	35	≥936	≥312	36	POP-4	60–400	≥420	<0.15	NA	NA	<1 bp	NA	N/A
SNaPshot50_POP7	30	≥1,104	≥376	50	POP-7	40–120	≥120	<0.50	NA	NA	<1 bp	NA	NA

NA = specification not applicable for this parameter.

1. Throughput (samples/day) is determined by the total number of samples that can be run in 23 hours (allows time for sample preparation, instrument maintenance, and warm-up).
2. Resolution range is defined as the range of bases over which the peak spacing interval divided by the peak width at half peak height is greater than 1.
3. Sizing precision is the standard deviation of sizes for a given allele size across all capillaries in the same run.
4. Multirun sizing is a measure of the precision of the 3500 Series across multiple runs. For example, it would be expected that a 200 bp allele across 3 runs would have an average deviation of <1 bp in 90% of all samples.

Find out more at **thermofisher.com/3500dx**

ThermoFisher SCIENTIFIC

Thermo Scientific molecular biology workflow solutions

High-quality essentials for everyday applications

ThermoFisher SCIENTIFIC

Supporting great science through innovation in molecular biology

For over two decades, the Thermo Scientific™ molecular biology portfolio has represented leading technology, enabling reliable performance for every step of the traditional molecular biology workflow. Our innovations include the first single-buffer restriction enzyme collection, the most widely used high-fidelity DNA polymerases, and the most comprehensive selection of PCR plastic consumables.

Today, the people behind our expanding portfolio remain committed to developing tools that deliver the best value for your research, with the performance and affordability that make it easy for you to do more great science.

To learn more, go to
thermofisher.com/thermoscientificmolbio

Contents

Nucleic acid isolation kits

High yields and exceptional value

Thermo Scientific™ GeneJET™ DNA and RNA purification kits are designed for rapid, efficient, and convenient purification of DNA and RNA from a wide range of samples. The kits utilize a proprietary 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. Purified DNA or RNA is ready to use in all common molecular biology procedures.

- Efficient nucleic acid extraction and high yields
- High purity of isolated DNA or RNA
- Simple and fast isolation procedure
- Convenient silica-based spin column format

Category	Description	Size	Cat. No.
Plasmid DNA purification	GeneJET Plasmid Miniprep Kit	50 preps/250 preps	K0502/K0503
	GeneJET Plasmid Midiprep Kit	25 preps/100 preps	K0481/K0482
	GeneJET Plasmid Maxiprep Kit	10 preps/25 preps	K0491/K0492
	GeneJET Endo-Free Plasmid Maxiprep Kit	10 preps	K0861
DNA and RNA fragment purification	GeneJET Gel Extraction Kit	50 preps/250 preps	K0691/K0692
	GeneJET PCR Purification Kit	50 preps/250 preps	K0701/K0702
	GeneJET RNA Cleanup and Concentration Micro Kit	50 preps/250 preps	K0841/K0842
	GeneJET Gel Extraction and DNA Cleanup Micro Kit	50 preps/250 preps	K0831/K0832
Genomic DNA purification	GeneJET Genomic DNA Purification Kit	50 preps/250 preps	K0721/K0722
	GeneJET Plant Genomic DNA Purification Mini Kit	50 preps/250 preps	K0791/K0792
	GeneJET Whole Blood Genomic DNA Purification Mini Kit	50 preps/250 preps	K0781/K0782
	GeneJET FFPE DNA Purification Kit	50 preps/250 preps	K0881/K0882
	GeneJET RNA Purification Kit	50 preps/250 preps	K0731/K0732
Total RNA purification	GeneJET Plant RNA Purification Mini Kit	50 preps/250 preps	K0801/K0802
	GeneJET Whole Blood RNA Purification Mini Kit	50 preps	K0761
	GeneJET Stabilized and Fresh Whole Blood RNA Kit	50 preps	K0871

To learn more, go to **thermofisher.com/genejet**

Reverse transcriptases

For optimal cDNA synthesis performance

Thermo Scientific™ Maxima™ reverse transcriptases (RTs) were developed through molecular evolution, which enabled the introduction and selection of multiple favorable mutations in traditional M-MuLV reverse transcriptase, boosting performance in cDNA synthesis. Maxima RTs are available in multiple formulations supporting a variety of molecular biology applications.

- Superior yields of full-length cDNA
- High reaction temperatures for improved transcription
- High transcription efficiency on long RNA templates
- Formats with integrated gDNA removal step for simplified workflows

Format	Description	Size	Cat. No.
Reverse transcriptases	Maxima Reverse Transcriptase	2,000 U/10,000 U	EP0741/EP0742
	Maxima H Minus Reverse Transcriptase	2,000 U/10,000 U	EP0751/EP0752
cDNA synthesis kits	Maxima First Strand cDNA Synthesis Kit for RT-qPCR	50 rxns/200 rxns	K1641/K1642
	Maxima First Strand cDNA Synthesis Kit for RT-qPCR, with dsDNase	50 rxns/200 rxns	K1671/K1672
	Maxima H Minus First Strand cDNA Synthesis Kit	20 rxns/100 rxns	K1651/K1652
	Maxima H Minus First Strand cDNA Synthesis Kit, with dsDNase	20 rxns/100 rxns	K1681/K1682
dsDNA synthesis kits	Maxima H Minus Double-Stranded cDNA Synthesis Kit	10 rxns	K2561

To learn more, go to **thermofisher.com/maxima**

For routine cDNA synthesis performance

Thermo Scientific™ RevertAid™ reverse transcriptases are based on M-MuLV enzymes and offer routine cDNA synthesis performance in molecular biology applications.

Format	Description	Size	Cat. No.
Reverse transcriptases	RevertAid Reverse Transcriptase	10,000 U/50,000 U	EP0441/EP0442
	RevertAid H Minus Reverse Transcriptase	10,000 U/50,000 U	EP0451/EP0452
cDNA synthesis kits	RevertAid First Strand cDNA Synthesis Kit	20 rxns/100 rxns	K1621/K1622
	RevertAid H Minus First Strand cDNA Synthesis Kit	20 rxns/100 rxns	K1631/K1632

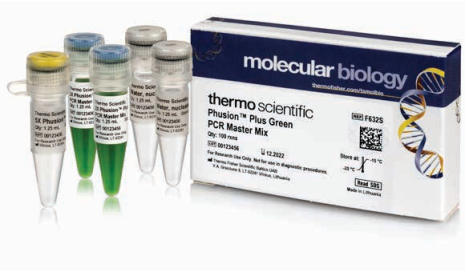
To learn more, go to **thermofisher.com/thermoscientificrt**

For reliable RNA protection

Thermo Scientific™ RiboLock™ RNase Inhibitor is an engineered thermostable enzyme that inhibits the activity of RNases A, B, and C. The enzyme is active under a wide range of reaction conditions and protects RNA at temperatures up to 55°C, helping to ensure successful reverse transcription in RT-PCR and RT-qPCR applications.

DNA polymerases

Trusted performance for high-fidelity PCR



Thermo Scientific™ Phusion™ high-fidelity DNA polymerases are designed to amplify DNA fragments with exceptional robustness and fidelity. Among the available Phusion formats, Thermo Scientific™ Phusion™ Plus DNA Polymerase allows you to skip calculation of annealing temperatures due to its universal annealing feature.

- High fidelity—Phusion Plus DNA Polymerase is >100x more accurate than *Taq* DNA polymerase
- Convenient—simplified PCR preparation and cycling with Phusion Plus DNA Polymerase due to a universal annealing temperature of 60°C

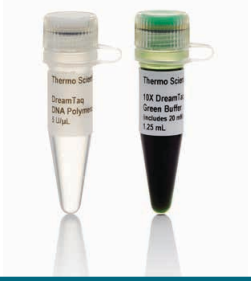
Green formats for Phusion and DreamTaq polymerases enable direct loading of PCR products on gels.

Format	Description	Size	Cat. No.
Standard	Phusion High-Fidelity DNA Polymerase	100 U/500 U	F530S/F530L
	Phusion High-Fidelity PCR Master Mix with HF Buffer	100 x 50 µL rxns/500 x 50 µL rxns	F531S/F531L
Hot-start	Phusion Hot Start II High-Fidelity DNA Polymerase	100 U/500 U	F549S/F549L
	Phusion Hot Start II High-Fidelity PCR Master Mix	100 x 50 µL rxns/500 x 50 µL rxns	F565S/F565L
	Phusion Plus DNA Polymerase	100 rxn/500 rxn	F630S/F630L
	Phusion Plus PCR Master Mix	100 rxn/500 rxn	F631S/F631L
	Phusion Plus Green PCR Master Mix	100 rxn/500 rxn	F632S/F632L
Uracil-tolerant	Phusion U Hot Start DNA Polymerase	100 U/500 U	F555S/F555L
	Phusion U Hot Start PCR Master Mix	100 x 50 µL rxns/500 x 50 µL rxns	F533S/F533L
Multiplex PCR	Phusion U Multiplex PCR Master Mix	100 x 50 µL rxns/500 x 50 µL rxns	F562S/F562L

To learn more, go to **thermofisher.com/phusion**

Enhanced *Taq* DNA polymerases for routine PCR

Thermo Scientific™ DreamTaq™ DNA polymerases offer a great balance between performance and value. Available in standard and hot-start formats, they deliver enhanced PCR performance that no conventional *Taq* enzyme can match.



- Featuring increased sensitivity and specificity; minimized optimization; and support of a wide range of amplicon lengths
- Multiple formats for maximum flexibility and reliability

Format	Description	Size	Cat. No.
Standard	DreamTaq DNA Polymerase	500 U/2,500 U	EP0702/EP0703
	DreamTaq Green DNA Polymerase	500 U/2,500 U	EP0712/EP0713
	DreamTaq PCR Master Mix	200 x 50 µL rxns/1,000 x 50 µL rxns	K1071/K1072
	DreamTaq Green PCR Master Mix	200 x 50 µL rxns/1,000 x 50 µL rxns	K1081/K1082
Hot-start	DreamTaq Hot Start DNA Polymerase	200 U/500 U/2,500 U	EP1701/EP1702/EP1703
	DreamTaq Hot Start Green DNA Polymerase	200 U/500 U/2,500 U	EP1711/EP1712/EP1713
	DreamTaq Hot Start PCR Master Mix	200 rxns/1,000 rxns	K9011/K9012
	DreamTaq Hot Start Green PCR Master Mix	200 rxns/1,000 rxns	K9021/K9022

To learn more, go to **thermofisher.com/dreamtaq**

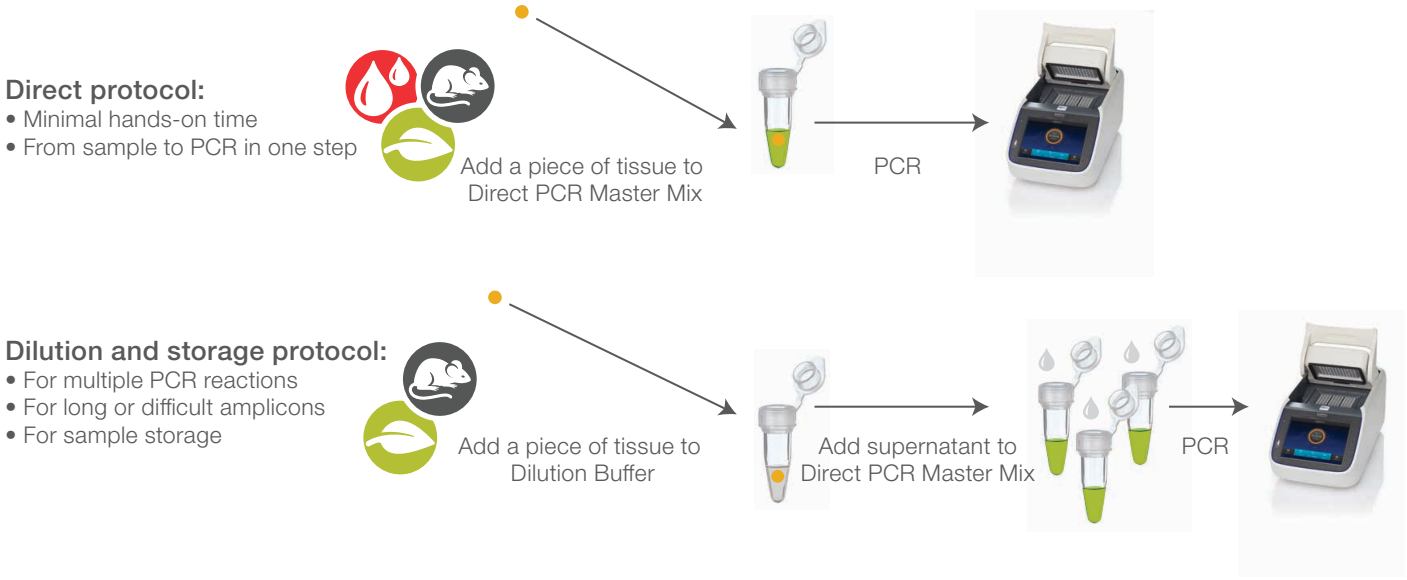
Solutions for direct PCR

Amplify without purification

Thermo Scientific™ Direct PCR master mixes offer outstanding convenience for DNA amplification by supporting PCR from unpurified samples. A tiny amount of source material is used in the PCR reaction without any purification steps, providing significant savings in both time and cost. Master mixes include a density reagent and two tracking dyes that allow for direct loading of PCR products on gels.

- PCR from crude samples—no DNA extraction or purification required
- Very short protocol times—from sample to results in 30 minutes
- Direct loading of PCR products on gels for simplified workflows
- Compatible with a variety of human, animal, and plant tissue samples

Two short protocols for different needs



Sample type	Description	Size		Cat. No.
		Direct protocol	Dilution and storage protocol	
Animal and human tissues	Phire Tissue Direct PCR Master Mix	100 rxns/500 rxns	250 rxns/1,250 rxns	F170S/F170L
Plant tissues, bacteria, yeast	Phire Plant Direct PCR Master Mix	100 rxns/500 rxns	250 rxns/1,250 rxns	F160S/F160L
Animal and human blood	Phusion Blood Direct PCR Master Mix	100 rxns/500 rxns	NA	F175S/F175L

To learn more, go to **thermofisher.com/directpcr**

PCR plastic consumables

Not all PCR plastics are created equal

For over 25 years, the Thermo Scientific™ PCR portfolio has been supplying high-quality PCR plastic consumables for molecular biology research. These products are designed to support maximum PCR performance and are manufactured with robust processes and extensive quality controls. The comprehensive portfolio of Thermo Scientific PCR plastic consumables includes individual tubes, tube strips, 96- and 384-well plates, and sealing options compatible with a broad range of PCR and qPCR instruments.

- Clean room production—certified free from DNA, RNases, and DNases
- Specialized solutions for low-, medium-, and high-throughput PCR and qPCR experiments
- Broad PCR and qPCR instrument compatibility including automated platforms
- Barcoded product options

Tubes
Individual PCR tubes with attached caps

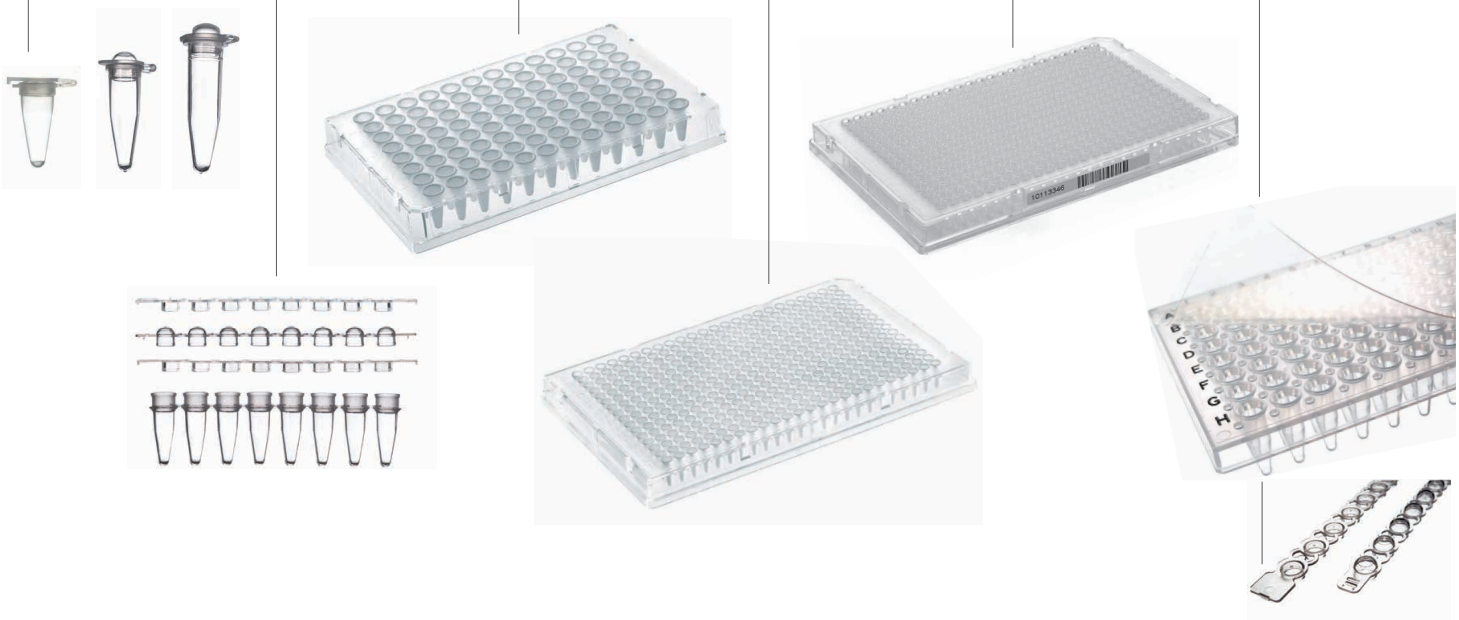
Tube strips
8-tube strips for PCR with separate or attached caps

96-well plates
Full-, semi-, or non-skirted 96-well PCR plates with different profiles; ultra-rigid plates for robotic applications

384-well plates
Full-skirted standard or extra-volume 384-well PCR plates; ultra-rigid plates for robotic applications

Barcoded plates
Barcoded 96- or 384-well PCR plates for reliable sample tracking

Sealing options
Cap strips, adhesive seals, and sealing mats compatible with PCR and qPCR applications



To learn more, go to **thermofisher.com/thermoscientificplastics**

Electrophoresis reagents

DNA ladders designed with accuracy in mind

Thermo Scientific™ GeneRuler™ DNA ladders are produced from chromatography-purified individual DNA fragments and are used for accurate analysis of DNA in agarose or polyacrylamide gels. They are ideal for sizing and in-gel DNA quantification. GeneRuler DNA ladders are available in conventional as well as ready-to-use formats (premixed with loading dye).

- Broad selection of DNA ladders that produce bright, sharp bands
- Environmentally friendly shipping

Range, bp*	Description	Size	Cat. No.
250–10,000	GeneRuler 1 kb DNA Ladder	5 x 50 µg/25 x 50 µg	SM0311/SM0312
	GeneRuler 1 kb DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM0314/SM0313
75–20,000	GeneRuler 1 kb Plus DNA Ladder	5 x 50 µg/25 x 50 µg	SM1331/SM1332
	GeneRuler 1 kb Plus DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM1334/SM1333
100–1,000	GeneRuler 100 bp DNA Ladder	50 µg/5 x 50 µg	SM0241/SM0242
	GeneRuler 100 bp DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM0243/SM0244
100–3,000	GeneRuler 100 bp Plus DNA Ladder	50 µg/5 x 50 µg	SM0321/SM0322
	GeneRuler 100 bp Plus DNA Ladder, ready-to-use	50 µg/5 x 50 µg	SM0323/SM0324
50–1,000	GeneRuler 50 bp DNA Ladder	50 µg/5 x 50 µg	SM0371/SM0372
	GeneRuler 50 bp DNA Ladder, ready-to-use	50 µg	SM0373

* GeneRuler DNA ladders are also available in ultralow (10–300 bp), low (25–700 bp), and high (10,171–48,502 bp) ranges.

To learn more, go to **thermofisher.com/dnaladders**

RNA ladders for fragment sizing and in-gel quantification

Thermo Scientific™ RiboRuler™ RNA ladders are produced from chromatography-purified RNA transcripts and are free from degraded RNA or NTPs. They produce sharp bands of uniform intensity and have easy-to-remember band sizes and quantities allowing for RNA fragment sizing and approximate quantification. RiboRuler RNA ladders are available in conventional as well as ready-to-use formats (premixed with loading dye).

To learn more, go to **thermofisher.com/rnaladders**

High-quality agarose

Thermo Scientific™ TopVision™ Agarose is a highly purified DNase- and RNase-free agarose that comes in two melting point options (standard and low melting temperature) and two formats (powder and tablets).

- Suitable for DNA and RNA analysis
- Excellent gel transparency

Format	Description	Size	Cat. No.
Powder	TopVision Agarose	100 g/500 g	R0491/R0492
	TopVision Low Melting Point Agarose	25 g	R0801
Tablets	TopVision Agarose Tablets	200/1,000 tablets	R2801/R2802

To learn more, go to **thermofisher.com/topvision**

Restriction and modifying enzymes

Restriction digestion simplified

Thermo Scientific™ FastDigest™ enzymes are a line of restriction enzymes that are all 100% active in a single buffer. The universal Thermo Scientific™ FastDigest™ and FastDigest™ Green Buffers allow single, double, or multiple DNA digestion within 5–15 minutes, eliminating any need for buffer changes or subsequent DNA cleanup steps. Thermo Scientific™ DNA-modifying enzymes have 100% activity in this buffer as well. The FastDigest Green Buffer includes a density reagent and two tracking dyes that allow for direct loading of digestion reaction products on gels.

- 100% activity of all FastDigest enzymes in one buffer
- Complete DNA digestion in 5–15 minutes
- 100% buffer compatibility with downstream applications

FastDigest Value Pack

The Thermo Scientific™ FastDigest™ Value Pack (Cat. No. K1991) is a collection of 13 popular FastDigest enzymes supplied with FastDigest and FastDigest Green Buffers. Each enzyme is supplied in an amount sufficient for 20 standard restriction digestion reactions. The FastDigest enzymes included in the pack are: BamHI, BglII, EcoRI, EcoRV (Eco321), HindIII, KpnI, NdeI, NotI, PstI, SalI, SmaI, XbaI, and XhoI.

Find all 176 enzymes at **thermofisher.com/fastdigest**

DNA- and RNA-modifying enzymes

Thermo Scientific™ modifying enzymes are of high quality and purity, and support common modifications of RNA and DNA molecules. These enzymes include phosphatases, kinases, DNA and RNA polymerases, ligases, and other nucleases.

Enzyme type	Description	Size	Cat. No.
Phosphatases and kinases	FastAP Thermosensitive Alkaline Phosphatase (1 U/µL)	1,000 U/5 x 1,000 U/300 U	EF0651/EF0652/ EF0654
	T4 Polynucleotide Kinase (10 U/µL)	500 U/2,500 U	EK0031/EK0032
DNA polymerases	T4 DNA Polymerase (5 U/µL)	100 U/500 U	EP0061/EP0062
	T7 DNA Polymerase (10 U/µL)	300 U	EP0081
	Klenow Fragment (10 U/µL)	300 U/1,500 U	EP0051/EP0052
Deoxyribonucleases (DNases)	Exonuclease I (20 U/µL)	4,000 U/20,000 U	EN0581/EN0582
	DNase I, RNase-free (1 U/µL)	1,000 U	EN0521
Ligases	T4 DNA Ligase (5 U/µL)	200 U/1,000 U	EL0014/EL0011
RNA polymerases	T7 RNA Polymerase, HC (200 U/µL)	25,000 U	EP0113
Ribonucleases (RNases)	RNase A, DNase- and protease-free (10 mg/mL)	10 mg	EN0531
	RNase H (5 U/µL)	100 U/500 U	EN0201/EN0202

Find all modifying enzymes at **thermofisher.com/tsmodifyingenzymes**

Cloning kits

Universal cloning kit for any type of DNA fragment

The Thermo Scientific™ CloneJET™ PCR Cloning Kit utilizes positive selection for fast and simple cloning. This kit supports highly efficient cloning of PCR products generated with any thermostable DNA polymerase and allows both blunt- or sticky-end phosphorylated or non-phosphorylated DNA fragments to be cloned.

- Fast—ligation in only 5–10 minutes
- High efficiency—more than 99% positive clones
- No cloning background with the positive selection vector
- Eliminates the need for blue/white screening

To learn more, go to **thermofisher.com/clonejet**

Ligation-independent cloning kits

Streamline and facilitate the process of cloning an insert into an expression vector with the Thermo Scientific™ aLICator™ LIC Cloning and Expression System. The included pLATE bacterial expression vectors are designed for high levels of target protein expression as well as minimized basal (uninduced) expression.

- No need to cut and ligate DNA with traditional methods
- Tight control for protein production
- One-step on-column His-tag removal

To learn more, go to **thermofisher.com/alicator**

Kits for DNA ligation and end repair

The Thermo Scientific™ Rapid DNA Ligation Kit enables fast sticky-end or blunt-end DNA ligation in only 5 minutes at room temperature. The fast ligation efficiency is equal to that obtained with T4 DNA ligase in a standard 1-hour ligation. The reaction mixture can be used directly for bacterial transformation.

The Thermo Scientific™ Fast DNA End Repair Kit is used for blunting and phosphorylation of DNA ends in just 5 minutes for subsequent use in blunt-end ligation.

Cloning kit	Description	Size	Cat. No.
Universal cloning kit	CloneJET PCR Cloning Kit	20 rxns/40 rxns	K1231/K1232
Ligation-independent cloning kits	aLICator LIC Cloning and Expression Kits	20 rxns	K1241, K1251, K1261, K1281
	aLICator LIC Cloning and Expression Systems	30 rxns	K1271, K1291
Kit for DNA ligation	Rapid DNA Ligation Kit	50 rxns/150 rxns	K1422/K1423
Kit for DNA end repair	Fast DNA End Repair Kit	50 rxns	K0771

To learn more, go to **thermofisher.com/cloningtools**

Visit our molecular biology resource library for webinars, videos, and articles at **thermofisher.com/mbresources**

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