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PureLink™ Quick Gel Extraction and PCR Purification Combo Kit

USER GUIDE

For purification of DNA fragments from agarose gels and rapid, efficient purification of PCR products

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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.

Revision history: MAN0001636 E (English)

Revision	Date	Description
E	9 April 2025	Rebranding and update of column storage recommendations.
D	13 July 2011	Baseline for revision history.

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

Contents

1 Product information

Product description

Use the PureLink™ Quick Gel Extraction and PCR Purification Combo Kit is used to rapidly and efficiently purify DNA fragments from agarose gels and from PCR or restriction enzyme reactions with silica membrane-based PureLink™ Clean-up Spin Columns.

The gel extraction protocol is used to purify DNA from TAE or TBE agarose gels of various percentages, and with different melting points in ~30 minutes by centrifugation or using a vacuum manifold.

The PCR purification protocol is used to efficiently remove primers, dNTPs, enzymes, and salts from PCR products in less than 15 minutes. The purified PCR product is suitable for DNA sequencing, cloning, restriction enzyme digestion, and labeling.

Kit specifications

Parmeter	Specification
Starting Material	≤400 mg agarose gel slice, or 50–100 µL PCR product (50 ng–40 µg dsDNA)
Elution Volume	50 µL
Separation Range	Separates 0.1–12 kb from 10–40 mer primers
DNA Fragment Size	40 bp–10 kb
DNA Recovery	• >80% for gel extraction • Up to 95% for PCR purification (dependant on DNA fragment size)
Primer Removal:	>99%
Binding Capacity	15 µg dsDNA per column
Column Reservoir Capacity	800 µL
Wash Tube Capacity	2.0 mL
Elution Tube Capacity	1.7 mL
Centrifuge Compatibility	>10,000 × *g*

Contents and storage

All components of the PureLink™ Quick Gel Extraction and PCR Purification Combo Kit are shipped at room temperature. Upon receipt, store all components at room temperature.

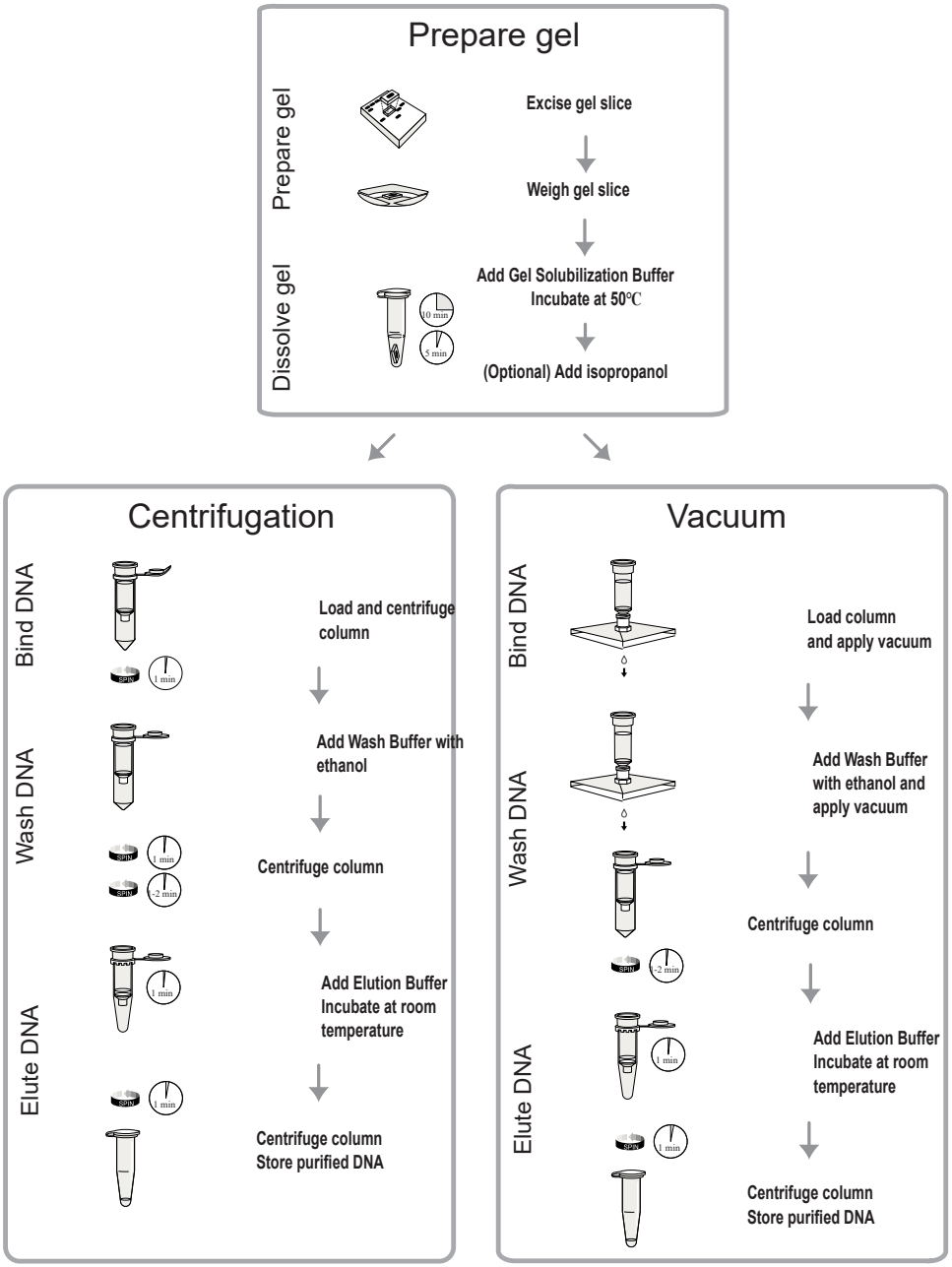
Note: For better long-term performance store PureLink™ Clean-up Spin Columns (in Wash Tubes) at 2°C to 8°C.

Component	Cat. no. K220001 (50 reactions)
Gel Solubilization Buffer (L3)	2 × 90 mL
Binding Buffer (B2)	15 mL
Wash Buffer (W1)	16 mL
Elution Buffer (E1) (10 mM Tris-HCl, pH 8.5)	15 mL
PureLink™ Clean-up Spin Columns (in Wash Tubes)	50 each
PureLink™ Elution Tubes	50 each

Experimental workflow

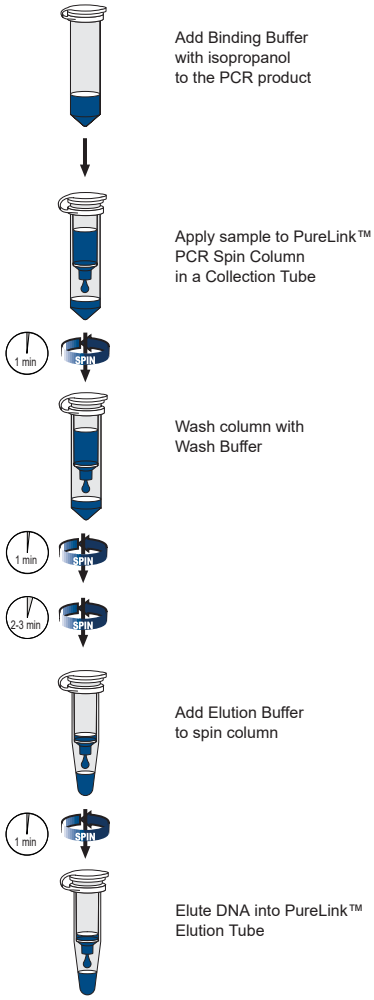
Gel extraction purification workflow

To purify DNA fragments from agarose gel using the PureLink™ Quick Gel Extraction and PCR Purification Combo Kit, excise the desired band and dissolve the TAE or TBE agarose gel. Place the dissolved gel into the Quick Gel Extraction Column to bind the DNA to a silica membrane, then purify and elute the DNA fragment by centrifugation or vacuum protocol.



PCR purification workflow

To purify PCR products using the PureLink™ Quick Gel Extraction and PCR Purification Combo Kit, mix PCR product with Binding Buffer to allow the dsDNA to bind to the silica-based membrane of the PureLink™ Spin Column. Add Wash Buffer and centrifuge to remove impurities, and elute purified DNA in low salt Elution Buffer or water.



2 Methods

Before first use of the kit

Before beginning, prepare Binding Buffer (B2) with isopropanol and Wash Buffer (W1) with ethanol according to the following directions.

Add isopropanol to Binding Buffer (B2)

1. Add 10 mL of 100% isopropanol to the bottle of Binding Buffer (B2).
2. Mark the checkbox on the Binding Buffer label to indicate that isopropanol was added to the bottle.
3. Store the Binding Buffer with isopropanol at room temperature.

Add ethanol to Wash Buffer (W1)

1. Add 64 mL of 96–100% ethanol to the bottle of Wash Buffer (W1).
2. Mark the checkbox on the Wash Buffer label to indicate that ethanol was added to the bottle.
3. Store the Wash Buffer with ethanol at room temperature.

Gel extraction protocol

The following protocol is used to purify DNA fragments from various percentage TAE and TBE agarose gels and from agarose gels with different melting points.

- The PureLink™ Quick Gel Extraction and PCR Purification Combo Kit is suitable for purifying DNA fragments from 40 bp to 10 kb in size. You may purify larger DNA fragments from gels using these kits; however, this may result in lower DNA recovery.
- The PureLink™ Quick Gel Extraction and PCR Purification Combo Kit is **not** designed to purify supercoiled plasmid DNA or genomic DNA from agarose gels. **Only linear DNA fragments can be purified from gels using these kits.**
- For best results, use one Quick Gel Extraction Column per 10 µg of DNA fragment loaded onto the gel, but up to 15 µg of DNA can be purified with each column. Use additional Quick Gel Extraction Columns if larger amounts of DNA are to be purified.

Prepare the gel slice

General guidelines

- Ensure that the DNA fragment of interest is **completely** separated from other DNA fragments on the agarose gel.
- To minimize DNA degradation, always wear gloves and use a clean razor blade to cut the gel slice.
- Maintain a sterile working environment when handling DNA to avoid any contamination from DNases.
- Ensure that no DNase is introduced into the sterile solutions supplied with the kit.
- Ensure that all equipment that comes in contact with DNA is sterile, including pipette tips and tubes.

CAUTION!

- The Gel Solubilization Buffer (L3) contains guanidine isothiocyanate (an irritant). This chemical is harmful when in contact with the skin, or when it is inhaled or ingested.
- **Do not** add bleach or acidic solutions directly to solutions or sample preparation waste that contains guanidine isothiocyanate, as reactive compounds and toxic gases are formed.

Always wear a laboratory coat, gloves, and safety glasses when handling this chemical. Dispose of the buffer and chemicals in appropriate waste containers.

Required materials not supplied

- Agarose gel containing the DNA fragment
- Weighing paper or weigh trays
- Scale (sensitive to 0.001 g)
- Water bath or heat block set at 50°C
- 1.5-mL or 5 mL-polypropylene microcentrifuge tubes
- Clean, sharp razor blade
- (*Optional:*) 100% isopropanol

Excise the DNA band

After completion of agarose gel electrophoresis:

1. Excise the area of the gel with the desired DNA band using a clean, sharp razor blade. Minimize the amount of agarose surrounding the DNA band.

2. Weigh the gel slice containing the DNA using a scale sensitive to 0.001 g, then place up to 400 mg of the excised gel slice into a polypropylene microcentrifuge tube.

Item	≤2% agarose gels	>2% agarose gels
Microcentrifuge tube	1.5-mL tube	5-mL

Note: If the gel slice with the desired DNA exceeds 400 mg, cut the gel into smaller slices so that no one piece exceeds 400 mg. Place each additional gel slice created into a separate microcentrifuge tube.

3. Proceed to "Dissolve the gel".

Dissolve the gel

1. Add Gel Solubilization Buffer (L3). to the excised gel slice containing the DNA fragment ("Excise the DNA band" on page 11).

Component	≤2% agarose gels	>2% agarose gels
	3 volumes buffer:1 volume of gel	6 volumes buffer:1 volume of gel
Gel Solubilization Buffer (L3)	1.2 mL	2.4 mL

Note: For example, add 1.2 mL or 2.4 mL of Gel Solubilization Buffer for a 400 mg gel slice.

2. Incubate the tube(s) containing the excised gel slice at 50°C for at least 10 minutes in a water bath or heat block.

   Invert the tube by hand every 3 minutes to mix and ensure gel dissolution.

Note: High concentration gels (>2% agarose) or large gel slices can take longer than 10 minutes to dissolve.

3. Incubate the tube for an additional 5 minutes after the gel slice appears to have dissolved.

4. (*Optional:*) For optimal DNA yields, add 1 gel volume of isopropanol to the dissolved gel slice. Mix well.

5. Proceed to "Purify DNA by centrifuge" on page 12, or "Purify DNA by vacuum" on page 13.

Purify DNA by centrifuge

This procedure is designed for purifying DNA fragments in approximately 30 minutes using a centrifuge.

Required materials not supplied

You will need the following items:

- 96–100% ethanol
- Microcentrifuge capable of >10,000 × *g*
- DNase-free pipettes and tips

Bind DNA

1. Pipet the dissolved gel piece containing the DNA fragment of interest (see "Dissolve the gel") onto the center of a Quick Gel Extraction Column inside a Wash Tube.

   Note: Do **not** load >400 mg dissolved agarose per Quick Gel Extraction Column.

2. Centrifuge at >10,000 × *g* for 1 minute.

   Discard the flow-through and replace the Quick Gel Extraction Column into the Wash Tube.

Wash DNA

1. Add 500 µL Wash Buffer (W1), containing ethanol (see page 9) to the Quick Gel Extraction Column.
2. Centrifuge at >10,000 × *g* for 1 minute.

   Discard the flow-through and replace the column into the Wash Tube.
3. Centrifuge the column again at maximum speed for 1–2 minutes to remove any residual Wash Buffer and ethanol. Discard the Wash Tube and place the Quick Gel Extraction Column into a Elution Tube.

Elute DNA

1. Add 50 µL Elution Buffer (E1) to the center of the Quick Gel Extraction Column.
2. Incubate the column for 1 minute at room temperature.
3. Centrifuge the Column at >10,000 × *g* for 1 minute. Discard the Quick Gel Extraction Column.

   The Elution Tube contains the purified DNA.
4. Store the purified DNA (see "Store the purified DNA" on page 14), or proceed to downstream application of choice.

Purify DNA by vacuum

This procedure is designed for purifying DNA fragments in approximately 30 minutes using a vacuum manifold. **Perform all vacuum operations at room temperature.**

Required materials not supplied

You will need the following items:

- 96–100% ethanol
- Vacuum manifold (with vacuum source capable of producing pressure of 15–20 in. Hg or –500 to –700 mbar)
- DNase-free pipettes and tips

Bind DNA

1. Assemble the vacuum manifold according to the manufacturer's instructions.
2. Attach a Quick Gel Extraction Column to the vacuum manifold.
3. Pipet the dissolved gel piece containing the DNA fragment of interest (see "Dissolve the gel") onto the center of the silica membrane of the Quick Gel Extraction Column. Apply the vacuum until all of the liquid passes through the column, then switch off the vacuum source.

Note: Do not load more than 400 mg dissolved agarose per column.

Wash DNA

1. Add 500–700 µL Wash Buffer (W1) containing ethanol (see page 9) to the center of the column.
2. Apply the vacuum until all of the liquid passes through the column, then switch off the vacuum. Remove the Quick Gel Extraction Column from the vacuum and place it into a Wash Tube.
3. Centrifuge the column with the Wash Tube at maximum speed for 1–2 minutes to remove any residual Wash Buffer and ethanol. Discard the flow-through and the Wash Tube. Place the Quick Gel Extraction Column into a Elution Tube.

Elute DNA

1. Add 50 µL Elution Buffer (E1) to the center of the Quick Gel Extraction Column.
2. Incubate the column for 1 minute at room temperature.
3. Centrifuge the Column at >10,000 × *g* for 1 minute. Discard the Quick Gel Extraction Column.
   The Elution Tube contains the purified DNA.
4. Store the purified DNA (see "Store the purified DNA" on page 14), or proceed to downstream application of choice.

PCR purification protocol

The procedure is used to purify up to 15 µg dsDNA using a centrifuge in a total time of 10–12 minutes.

Bind DNA

1. Add 4 volumes of Binding Buffer (B2) with isopropanol (see page 9) to 1 volume of the PCR product (50–100 µL). Mix well.
2. Remove a PureLink™ Clean-up Spin Column in a Wash Tube from the package.
3. Add the diluted PCR sample to the PureLink™ spin column.
4. Centrifuge the column at room temperature at >10,000 × *g* for 1 minute.
5. Discard the flow through and place the spin column into the wash tube.

Wash DNA

1. Add 650 µL of Wash Buffer with ethanol (see page 9) to the spin column.
2. Centrifuge the column at room temperature at >10,000 × *g* for 1 minute. Discard the flow through from the wash tube and place the column back into the tube.
3. Centrifuge the column at maximum speed at room temperature for 2–3 minutes to remove any residual Wash Buffer. Discard the wash tube.

Elute DNA

1. Place the spin column in a clean 1.7-mL PureLink™ Elution Tube (supplied with the kit).
2. Add 50 µL of Elution Buffer (E1) or sterile, distilled water (pH >7.0) to the center of the column.
3. Incubate the column at room temperature for 1 minute.
4. Centrifuge the column at maximum speed for 1 minute.
5. *The elution tube contains the purified PCR product.* Remove and discard the column. The recovered elution volume is ~48 µL.

Store the purified DNA

Store the purified DNA at 4°C for immediate use, or prepare aliquots of the DNA and store at –20°C for long-term storage. Avoid repeated freeze thaw cycles of the DNA.

Estimate DNA yield and quality

After DNA purification, determine the quantity and quality of the purified DNA.

Determine DNA yield

Measure the DNA concentration by UV absorbance at 260 nm, agarose gel electrophoresis, or Qubit™ DNA Assay Kits.

Determine DNA yield with Qubit™ DNA Assay Kits

Measure DNA concentration using Qubit™ DNA Assay Kits or UV absorbance at 260 nm.

The Qubit™ DNA Assay Kits (see "Additional products" on page 19 for ordering information) provide a rapid, sensitive, and specific method for measuring dsDNA concentration with minimal interference from RNA, protein, ssDNA (primers), or other common contaminants that affect UV absorbance.

The kit contains a state-of-the-art quantitation reagent, pre-diluted standards for standard curve, and a pre-made buffer. For optimal results, perform the quantitation using a Qubit™ 4 Fluorometer (see "Additional products" on page 19). You can also use standard fluorescent microplate readers. Follow manufacturer's recommendations to perform the assay.

Determine DNA yield by agarose gel electrophoresis

Perform agarose gel electrophoresis with the purified PCR product and known quantities of DNA fragments of the same size. Compare the band intensities of the purified PCR product with the standard DNA fragments. The band intensity of the known standard that approximates the band intensity of the PCR product provides an estimate of the DNA yield.

Determine yield by UV absorbance

1. Prepare a dilution of the DNA solution in 10 mM Tris-HCl, pH 7.5. Mix well. Measure the absorbance at 260 nm (A_{260}) of the dilution (using a cuvette with an optical path length of 1 cm) in a spectrophotometer blanked against 10 mM Tris-HCl, pH 7.5.

2. Calculate the concentration of DNA using the following formula.

   DNA (µg/mL) = A_{260} × 50 × dilution factor

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

Note: Contaminating RNA will inflate the DNA content measured at 260 nm. To avoid any interference from RNA, use the Qubit™ DNA Assay Kits to measure DNA concentration.

Expected results

DNA fragments of various sizes were purified using PureLink™ Quick Gel Extraction and PCR Purification Combo Kit as described in the manual. The concentration of purified DNA was measured using Qubit™ DNA Assay Kits (see "Additional products" on page 19 for ordering information). The DNA yields are listed in the table.

DNA Size	Amount Loaded	% DNA Yield[1]
400 bp	0.5 µg	>85%
740 bp	1 µg	>85%
2.9 kb	1 µg	>85%

[1] **Note:** The DNA yield varies with the fragment size, amount of DNA loaded on the gel, gel slice size, elution volume, and incubation time.

Estimate DNA quality

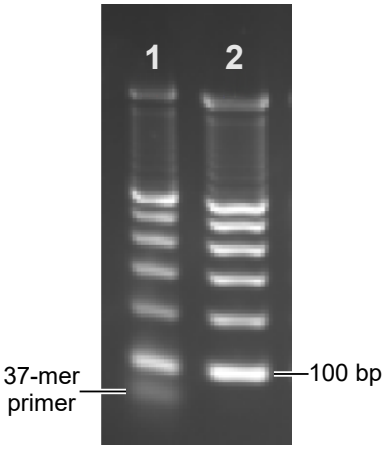
Typically, DNA isolated using the PureLink™ Quick Gel Extraction Kit has an A_{260}/A_{280} >1.8 when samples are diluted in Tris-HCl pH 7.5–8.5 indicating that the DNA is substantially free of contaminants that would otherwise affect UV absorbance.

To confirm the integrity and the size of the purified DNA fragment, perform agarose gel electrophoresis.

Estimate primer removal

Analyze the efficiency of primer removal by agarose gel electrophoresis. An example is shown in the following section.

A 100 bp DNA Ladder (Cat. no. 15628-019) sample spiked with an excess of a 37-mer primer was purified according to the protocol using Binding Buffer (B2). The spiked ladder and purified sample were analyzed using agarose gel electrophoresis, and shows removal of the primer.



- Lane 1: Sample before purification
- Lane 2: Sample after purification

A

Troubleshooting

Gel extraction troubleshooting

Observation	Cause	Solution
Low DNA yield	Incorrect ratio of gel to Gel Solubilization Buffer	Ensure that the correct volume of Gel Solubilization Buffer (L3) is added for every 1 volume of gel used, based on the agarose gel percentage (see "Dissolve the gel" on page 11).
	Incomplete solubilization of gel piece	Verify that the temperature of water bath or heat block is at 50°C. Cut large gel slices into several pieces to accelerate the gel dissolution. Mix gel slice in the buffer every 3 minutes during the dissolution step.
	DNA fragment is too large	Increase the incubation time for elution to >10 minutes.
	DNA is supercoiled	This kit is not designed to purify supercoiled plasmid DNA from agarose gels.
Low $A_{260/230}$ ratio	Guanidine carryover from the Gel Solubilization Buffer	• Do not get any buffer solution in the cap area of the tube. • Add a second wash step with Wash Buffer (W1): After your first wash with Wash Buffer, followed by centrifugation: **a.** Add another 500–700 µL Wash Buffer, containing ethanol. **b.** Centrifuge at >10,000 × g. Discard the flow-through and return the column into the Wash Tube. **c.** Centrifuge at maximum speed for 2–3 minutes to remove residual Wash Buffer and ethanol.
Downstream enzymatic reactions are inhibited	Residual ethanol in the purified DNA	Traces of ethanol from the Wash Buffer can inhibit downstream enzymatic reactions. To remove Wash Buffer, discard Wash Buffer flow through from the Wash Tube. Place the column into the Wash Tube and centrifuge the column at >10,000 × g for 2–3 minutes to completely dry the column.

PCR purification troubleshooting

Observation	Cause	Solution
Low DNA yield	PCR conditions not optimized	Check the amplicon on the gel to verify the PCR product prior to purification.
	Incorrect binding conditions	• For efficient DNA binding always mix 1 volume of PCR (50–100 µL) with 4 volumes of Binding Buffer. • Be sure to add 100% isopropanol to the Binding Buffer (see page 9).
	Ethanol not added to Wash Buffer	Be sure to add 96–100% ethanol to Wash Buffer (see page 9).
	Incorrect elution conditions	Add elution buffer to the center of the column and perform incubation for 1 minute with Elution Buffer before centrifugation.
Downstream enzymatic reactions are inhibited	Presence of ethanol in purified DNA	Traces of ethanol from the Wash Buffer can inhibit downstream enzymatic reactions. To remove Wash Buffer, discard Wash Buffer flow-through from the Wash Tube. Replace the column into the Wash Tube and centrifuge the column at >10,000 × *g* for 2–3 minutes to completely dry the column.

B

Related products

Additional products

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Product	Quantity	Catalog no.
PureLink™ Quick Gel Extraction Kit	50 preps 250 preps	K2100-12 K2100-25
PureLink™ PCR Purification Kit	50 reactions 250 reactions	K3100-01 K3100-02
EveryPrep™ Universal Vacuum Manifold	1 unit	K2111-01
Platinum™ Taq DNA Polymerase High Fidelity	100 reactions	11304-011
Platinum™ Taq DNA Polymerase	100 reactions	10966-018
UltraPure™ DNase/RNase-free Distilled Water	500 mL	10977-015
Qubit™ dsDNA Assay Kit, High Sensitivity	500 assays	Q32854
Qubit™ dsDNA Assay Kit, Broad-Range	500 assays	Q32853
Qubit™ 2.0 Fluorometer	1 each	Q32857

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 are available from Thermo Fisher Scientific for sizing DNA. For more details on these products, visit **thermofisher.com**.

C

Safety

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

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

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

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

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

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. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

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  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and its affiliates 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 questions, contact Life Technologies at **www.thermofisher.com/support**.