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VersaTaq™ Direct PCR Polymerase

Product number 71002

Storage
Store at -20°C.

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

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Components

All reagents have been extensively tested and carefully prepared to meet USB® standards. USB recommends the reagents be used as directed to achieve the best possible results.

The following components are included:

VersaTaq Direct PCR Polymerase: 1.0 µg/µl, determined by OD_{280}. Supplied in 20 mM Tris, pH 8.55, 222 mM ammonium sulfate, 10 mM 2-mercaptoethanol, 63% glycerol, 0.1 mM EDTA, and 0.1% Brij® 58.

10X VersaTaq Direct PCR Reaction Buffer: 500 mM Tris-HCl, pH 9.1, 160 mM ammonium sulfate, 25 mM $MgCl_2$, and 0.25% Brij 58.

Quality control

Enzyme purification test

A 94 kDa single band with approximately 95% homogeneity as determined by SDS-PAGE.

PCR amplification

A 2-fold serial dilution of VersaTaq Direct PCR Polymerase is performed and aliquots used in PCR to amplify a 2 kb fragment from 1 ng of lambda DNA in 26 cycles. Observed amplification at ⅛-fold dilution and comparable performance to a reference lot passes the test (50 µl reaction volume).

Inhibition resistance test

A 1 kb human target is amplified directly from 20% EDTA-treated blood using 0.5, 0.25, and 0.125 µl of VersaTaq Direct PCR Polymerase and 1X VersaTaq Direct PCR Reaction Buffer. PCR is performed for 35 cycles in the absence of enhancers or other additives. Observed amplification with 0.125 µl of enzyme passes the test (25 µl reaction volume).

E. coli DNA contamination test

Primers specific to a 346 bp fragment of *E. coli* 16S rRNA gene are used with VersaTaq Direct PCR Polymerase for PCR in the presence or absence of *E. coli* X7029 DNA template. After 35 cycles, amplification is observed in the positive control containing template and no amplification is observed in absence of *E. coli* X7029 DNA template.

Endonuclease contamination test

Incubation of 1.0, 0.5, and 0.25 µl of VersaTaq Direct PCR Polymerase with 500 ng of supercoiled phiX174 RF DNA in 1X VersaTaq Direct PCR Reaction Buffer for 4 hours at 37°C results in no change in band pattern compared to a no-enzyme control observed in agarose gel electrophoresis (50 µl reaction volume).

Safety warnings and precautions

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

All chemicals should be considered potentially hazardous. Read the MSDS (SDS) before handling or working with any chemical. This product should only be handled by those persons who have been trained in laboratory techniques and it should be used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as lab coat, safety glasses, and gloves. Care should be taken to avoid contact with skin and/or eyes. In the case of contact with skin or eyes, wash immediately with water. See the Safety Data Sheet for specific information regarding this product.

Description

VersaTaq Direct PCR Polymerase is a mutant of Taq DNA Polymerase selected for direct amplification from complex templates. It confers resistance to many potent PCR inhibitors such as those found in whole blood, soil, feces, urine, buccal swabs, animal tissue, and plant tissue. In contrast to standard Taq DNA Polymerase, VersaTaq Direct PCR Polymerase can amplify DNA directly from samples, which eliminates costly extraction and purification steps, saving time and enabling amplification from limited quantities of DNA. This versatile polymerase remains functional in the presence of high concentrations of inhibitors where other commercial enzymes fail. Due to the wide variety of sample types supported by VersaTaq Direct PCR Polymerase, optimization may be required. Enzyme titration experiments are recommended for numerous inhibitory sample types, and the addition of PCR enhancers such as betaine can significantly improve long target and high GC amplification. VersaTaq Direct PCR Polymerase also has 5′-3′ exonuclease activity making it suitable for hydrolysis probe-based detection chemistries.

This protocol describes the versatility of VersaTaq Direct PCR Polymerase with several common sample types and provides general recommendations for performing PCR.

Whole blood

Whole blood contains substances such as heme and a variety of proteins that have been shown to inhibit polymerase activity in PCR reactions[1-3]. The inability to amplify DNA in the presence of these inhibitors has led to problems for clinical and forensic applications. VersaTaq Direct PCR Polymerase overcomes PCR inhibitors in blood, enabling direct DNA amplification in as high as 40% whole blood. VersaTaq Direct PCR Polymerase is able to amplify DNA in whole blood with various anticoagulants from a wide range of targets including high GC (Fig. 1).

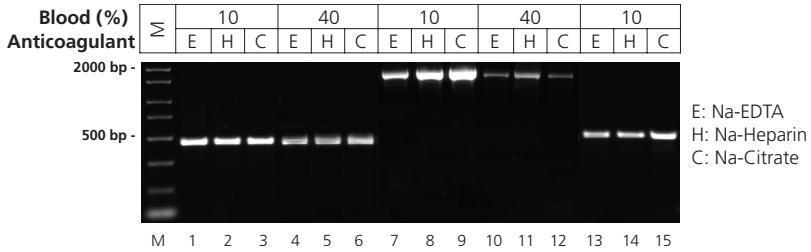

Figure 1. Amplification of targets directly from human whole blood. Targets were amplified from different concentrations of human whole blood in the presence of varying types of anticoagulants. Lanes 1-6: *NUMB* 455 bp; Lanes 7-12: *NUMB* 1.8 kb; Lanes 13-15: *HRES1* 488 bp - corresponding band is an 81% GC amplicon. 1 M betaine added to reactions with high GC targets.

Blood spots

VersaTaq Direct PCR Polymerase can amplify directly from dry blood spotted on Whatman™ FTA™ elute cards (Fig. 2). Using VersaTaq Direct PCR Polymerase decreases processing time significantly because there is no need to elute DNA from the cards prior to performing PCR.

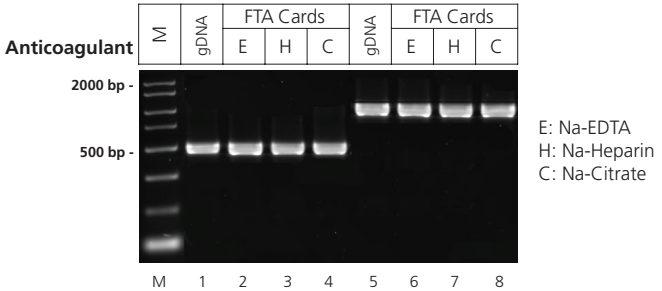

Figure 2. Amplification of targets from human whole blood spotted on Whatman FTA elute cards by VersaTaq Direct PCR Polymerase. *NUMB* gene was amplified from FTA cards spotted with human whole blood in the presence of varying types of anticoagulant and from genomic DNA control. 2 mm punches from FTA cards were placed directly into PCR reactions. Lanes 1-4: *NUMB* 455 bp; Lanes 5-8: *NUMB* 967 bp.

Buccal swabs

Buccal swabs for DNA collection can be used in a wide range of applications such as genotyping and epigenetic studies. This protocol describes a rapid elution technique for preparing DNA from buccal swabs that can be added directly to PCR reactions with VersaTaq Direct PCR Polymerase for amplification from a wide range of targets (Fig. 3).

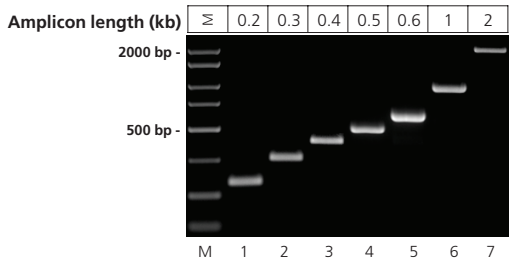

Figure 3. Amplification of targets directly from buccal swab eluent by VersaTaq Direct PCR Polymerase. Targets were amplified from buccal swabs (supplied by Isohelix, Kent, UK) eluted in 0.1% SDS and added directly into PCR reaction. Lane 1-7: *NUMB* 196 bp, 304 bp, 404 bp, 493 bp, 609 bp, 967 bp, 2 kb.

Soil

Environmental and forensic applications that require samples collected from crude soil for DNA amplification may present problems in PCR reactions. Trace amounts of humic acid commonly found in aquatic soil and sediment environments have been shown to inhibit PCR[4]. Taq DNA Polymerase is typically inhibited in the presence of less than 1 ng/µl of humic acid and successful PCR requires removal or attenuation from samples[5]. VersaTaq Direct PCR Polymerase overcomes potent inhibitors, enabling direct amplification from a wide range of targets including those with high GC content (Fig. 4).

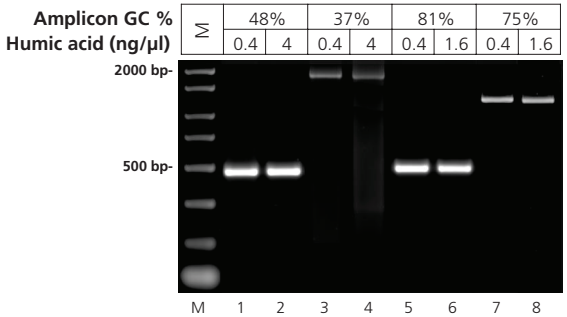

Figure 4. Amplification of diverse targets in presence of humic acid by VersaTaq Direct PCR Polymerase. Targets were amplified from 5 ng of genomic DNA in the presence of varying concentrations of humic acid. Lanes 1-2: *NUMB* 455 bp; Lanes 3-4: *NUMB* 1.8 kb; Lanes 5-6: *HRES1* 488 bp; Lanes 7-8: *SIM2* 1.2 kb. 1 M betaine added to reactions with high GC content.

Feces

Detecting microorganisms in human or animal feces using PCR is difficult due to the presence of PCR inhibitors such as bile salts. Taq DNA Polymerase is inhibited by 25 µg of bile salts[6], whereas VersaTaq Direct PCR Polymerase can amplify a wide range of targets including those with high GC content in the presence of three times this amount (Fig. 5). VersaTaq Direct PCR Polymerase can also amplify targets in the presence of crudely extracted feces (Fig. 5).

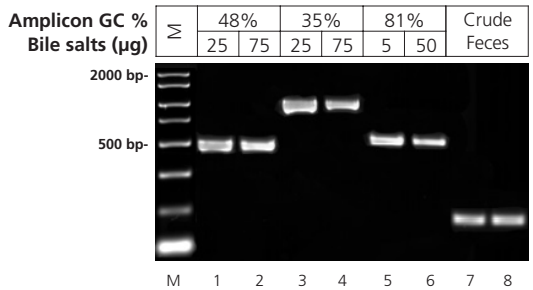

Figure 5. Amplification of targets in presence of bile salts by VersaTaq Direct PCR Polymerase. Targets were amplified from 5 ng of genomic DNA in the presence of varying concentrations of bile salts. Lanes 1-2: NUMB 455 bp; Lanes: 3-4 NUMB 967 bp; Lanes: 5-6 HRES1 488 bp. 1 M betaine soil added to reactions with high GC content. Lanes 7-8: 110 bp lambda targets were amplified from 5 ng of lambda DNA in the presence of crude extract feces. Lane 8 amplification was also in the presence of RNAlater®.

Urine

DNA detection in urine specimens, using PCR, is important for detection of biomarkers related to diseases and other health indicators[7]. Urine contains urea, which inhibits PCR at concentrations greater than 50 mM[8]. VersaTaq Direct PCR Polymerase can amplify DNA at urea concentrations far exceeding the amount found in typical adult urine, which is about 330 mM[9] (Fig. 6).

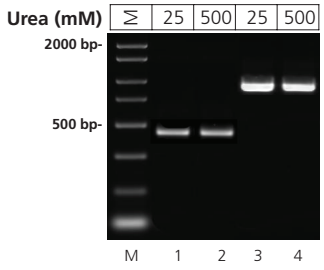

Figure 6. Amplification of targets in presence of urea by VersaTaq Direct PCR Polymerase. Targets were amplified from 5 ng of genomic DNA in the presence of varying concentrations of urea. Lanes 1-2: *NUMB* 455 bp; Lanes 3-4: *NUMB* 967 bp.

Animal tissue

Tissue such as that isolated from genetically modified mouse models are extensively used in biomedical research. PCR from tissue samples typically requires a DNA extraction step due to PCR inhibitors[10-11]. VersaTaq Direct PCR Polymerase overcomes PCR inhibitors commonly found in tissue, enabling direct amplification of a wide range of DNA targets (Fig. 7).

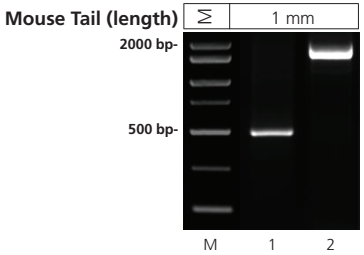

Figure 7. Amplification of targets directly from mouse tail by VersaTaq Direct PCR Polymerase. Targets were amplified from 1 mm mouse tail clip added directly into PCR reaction. Lane 1: *NUMB* 483 bp; Lane 2: *NUMB* 1.8 kb.

Plant tissue

With the emergence of genetically modified crops, PCR is becoming an even more important tool in agriculture to detect transgenes in the modified plant DNA. Plants carry many substances, such as polysaccharides, polyphenols, pectin, and xylan, which may inhibit PCR[12]. VersaTaq Direct PCR Polymerase can amplify targets from a small piece of plant added directly to the reaction (Fig. 8).

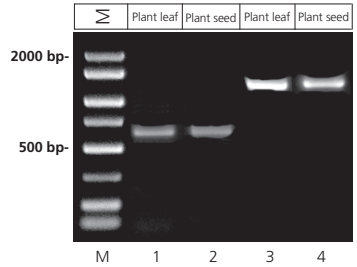

Figure 8. Amplification of targets directly from plant leaf and seed by VersaTaq Direct PCR Polymerase. Targets were amplified from 0.5 mm plant tissue added directly into PCR reaction. Lane 1-2: ABI37 672 bp; Lane 3-4: EIL5 1323 bp. Lane 1 and 3 directly from plant leaf, Lane 2 and 4 directly from plant seed.

Materials not supplied

Necessary reagents:

DNA template: PCR, both with purified DNA and directly from DNA within a diverse variety of sample types including blood, soil, feces, urine, tissue, etc., can be performed using VersaTaq Direct PCR Polymerase.

Oligonucleotide primers: Primers can be designed according to standard PCR methods[13]. Use common oligo design computer programs when possible.

PCR Nucleotide Mix, 10 mM Solution (PN 77212)

Water, Nuclease-Free (PN 71786)

Optional reagents:

Enhancing additives: Solvents such as dimethyl sulfoxide (DMSO), glycerol, trehalose, and betaine can improve results for longer targets, high GC content amplicons, and those with a high degree of secondary structure[14-17].

Necessary equipment:

Liquid handling supplies such as PCR-grade thin-walled tubes, pipettes, pipettors, and a microcentrifuge are required. Use barrier-tip pipettes and dedicated PCR pipettors to avoid contamination.

Latex gloves (powder-free) should be used for handling reagents and equipment in order to decrease the probability of introducing contaminates into samples.

Thermal cycler for incubations between 4°C and 95°C.

Equipment such as a standard horizontal gel apparatus and a UV transilluminator or fluorescence image scanner can be used for analysis of PCR products.

Protocol

This standard protocol applies to a single reaction. Master mixes for multiple reactions can be made by increasing the volumes of reaction components proportionally.

1. Thaw frozen reagents on ice. Mix thoroughly and then return to ice.
2. Assemble reaction tubes on ice whenever possible to avoid non-specific polymerase activity.

3. The following table shows recommended component volumes:

Components	25 µl reaction volume	50 µl reaction volume	Final concentration
10X VersaTaq Direct PCR Reaction Buffer	2.5 µl	5.0 µl	1X
10 mM PCR Nucleotide Mix (dNTPs)	0.5 µl	1.0 µl	0.2 mM each dNTP
10 µM Forward Primer	0.5 µl	1.0 µl	0.2 µM
10 µM Reverse Primer	0.5 µl	1.0 µl	0.2 µM
Template DNA/Tissue sample or extract	variable*	variable*	
VersaTaq Direct PCR Polymerase	0.05–0.25 µl**	0.1–0.5 µl**	
Water, PCR-Qualified	up to 25 µl	up to 50 µl	

*Input for 50 µl reaction (scale proportionately per reaction volume):

1. Purified DNA: <50 ng
2. Whole blood: ≤10 µl[a]
3. Blood spots stored on Whatman FTA elute cards: 2 mm punch[b]
4. Urine: ≤10 µl
5. Crude extracts from soil or feces: ≤5 µl[c]
6. Animal tissue: <1 mm
7. Buccal swab eluent: 5-15 µl[d]
8. Plant tissue: <0.5 mm

**Enzyme titration experiments may be required to optimize enzyme performance.

a) For whole blood:

A. Before adding blood, thoroughly mix components by pipetting up and down.
B. Add blood last and let settle to the bottom of the PCR tube. The reaction should not be mixed after the blood is added.
C. For reactions containing >20% blood, a 50 µl reaction volume is recommended.
Note: Although 40% blood (v/v) can be tolerated, 5%–20% is recommended. High concentrations of blood are not recommended due to difficulties in recovery of the aqueous supernatant from blood cellular debris that remains after the reaction. Spinning PCR tubes at 5,000 RPM for 3 minutes will facilitate separation of aqueous supernatant from remaining cellular debris.

b) For blood spots stored on Whatman FTA elute cards:

A. Incubate the 2 mm punch first in 50 µl PCR qualified water at 50°C for 5 minutes
B. Discard the 50 µl water and use the 2 mm punch directly in PCR.

c) For feces:

A. Homogenize fecal material until it is well suspended
Note: If detecting microorganisms such as gram positive bacteria or fungi, a sonication or bead beater step may lead to more efficient cell lysis.
B. Heat the suspension for 15 minutes at 70°C.
C. Quick spin at 1,000 RPM for 3 minutes, collect, and save supernatant.
D. Add 1-5 µl of supernatant directly into the PCR reaction.

d) For buccal swabs:

A. Swab cheek per vendor's protocol.
B. If swabs are not dry, allow swabs to dry at room temperature.
C. Remove the entire swab head and place into a microcentrifuge tube containing 500 µl of 0.1% SDS detergent solution.
D. Incubate swab/detergent solution at 70°C for 15 minutes followed by 95°C for 2 minutes.
E. Remove swab and use eluent directly in PCR or store at 4°C.

4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.

5. Transfer PCR tubes to a thermal cycler and use the following table for recommended cycling conditions:

Cycle step	Temperature	Time
Initial denature*	95°C	3-10 minutes
Denature	95°C	20 seconds
Anneal**	60°C	30 seconds
Extend†	68°C	2 minutes
Repeat previous three cycles as necessary, generally 25-35 times.		
Final extension	68°C	10 minutes
Final hold	4°C	as necessary

* Initial denature time when using:

1. Purified DNA: 3 minutes
2. Whole blood: 3 minutes
3. Blood spots stored on Whatman FTA elute cards: 5 minutes
4. Urine: 3 minutes
5. Crude extracts from soil or feces: 10 minutes
6. Animal tissue: 10 minutes; (see Note 1 below regarding Proteinase K)
7. Buccal swab: 10 minutes
8. Plant tissue: 10 minutes (see Note 2 below)

**Initially, the annealing temperature should be 5°C below the calculated T_m of the primers. If non-specific products are produced, increase the annealing temperature in 1-2°C increments.

† Extension time should be 2 minutes/kb for expected products.

6. Analyze sample (typically 1 to 10 µl aliquot) by agarose gel electrophoresis. Visualize PCR product in gel with a DNA intercalating dye and a UV transilluminator or fluorescence imager.
   Note 1: For animal tissue analysis, dilute Proteinase K (PN 76225) 1:10 in 6X DNA Loading Buffer. For example, add 5 µl of Proteinase K into 45 µl 6x DNA Loading Buffer. Then, add 15 µl of DNA Loading Buffer-Proteinase K mix into finished 50 µl PCR reactions (Proteinase K final concentration of 0.2 mg/ml). Mix reactions and incubate for 30-60 minutes at 50°C. Spin tubes and analyze sample by agarose gel electrophoresis.
   Note 2: For plant tissue, 1 M Betaine (PN 77507) is required as a PCR enhancer for successful amplification (10% DMSO can be used in place of Betaine).

Supplementary information

Success in PCR depends on many factors. Careful planning and experimental design, based on an understanding of these factors, can be crucial in obtaining the desired product quickly and in optimizing challenging reactions. This section serves as a starting point for understanding those factors. Additional information, such as new product listings, updated protocols, and Tech Tips, may be found at our website, usb.affymetrix.com

Primer design

General rules for designing PCR primers can be found in many texts[13]. In general, primers should range in length from 18 to 30 nucleotides, exhibit GC content similar to each other (and ideally in the range of 40% to 60%), and exhibit T_m values ranging from 55°C to 65°C and that are closely matched to each other. T_m values may be estimated using the following equation: $T_m(°C) = 2(A+T) + 4(G+C)$. More accurate methods for calculation of T_m values may also be applied[13]. Primers that do not fit these criteria may also function well, but empirical testing is required. Using computer programs designed to select appropriate primers is highly recommended. In addition, several public primer databases are available on the internet.

Suggestions for difficult templates

DNA products with GC rich regions (*e.g.*, >60%) and longer lengths (*e.g.*, >2 kb) in the presence of inhibitory matrices can be difficult to amplify. Adding certain supplements, such as DMSO, glycerol, trehalose, and/or betaine to the PCR reaction may dramatically improve results[14-17]. DMSO and glycerol may be added at final concentrations ranging from 1% to 10%[13]. Trehalose may be added to 0.6 M final concentration[14]. Betaine (PN 77507, 5 M Stock Solution) may be added at 0.5 M to 2.0 M final concentration[14, 17] Betaine and trehalose have been reported to thermostabilize proteins in general[14, 16] All of these solvents tend to decrease T_m values for double-stranded DNA, thus its presence in reactions may result in a decrease in the optimum annealing temperature by several degrees. Addition of supplemental magnesium chloride may also improve results. Benefits are usually observed within a narrow range and concentrations above 5 mM should be avoided.

Troubleshooting

Problem Possible causes and solutions

No product or faint product

1. Use more/less template of sample.
2. Increase number of cycles, up to 45 cycles for low-copy targets.
3. Increase the concentration of primers, for example from 0.2 µM to 0.4 µM.
4. Test a range of PCR annealing temperatures. Start with annealing temperature 5-10°C below primer T_m and increase in 1-2°C increments. Annealing temperatures that are either too high or too low can result in absence of product.
5. If template and/or primers exhibit GC content greater than ~60%, consider supplementing reactions with betaine or additives as suggested in the 'Supplementary information' section. In general, the optimal amount of additives for a given primer/template combination needs to be determined empirically.
6. For products longer than 2 kb or longer targets from complex DNA templates, consider using betaine or the additives as suggested in the 'Supplementary information' section.
7. Mix master mixes and individual reactions well (not including reactions involving blood). Spin down contents to bottom of tube.
8. Design new primers. Use oligo design computer program if at all possible.
9. Increase the initial denature time.

Nonspecific bands and/or background smearing

1. Use less DNA template/sample.
2. Reduce the amount of enzyme in the reaction.
3. Reduce number of cycles.
4. Raise annealing temperature in 1-2°C increments.
5. Decrease the concentration of primers, for example from 0.4 µM to 0.1 µM. Do not exceed 1 µM.
6. Design new primers. Longer oligonucleotides (*e.g.*, > 25 bases) and those with higher melting temperatures (*e.g.*, > 60°C) can achieve more specific amplification.
7. Supplement reactions with additives that improve amplification of GC rich templates or longer products such as betaine.

If problems persist please contact Technical Support for assistance at (888) 362-2447 or USBtechsupport@affymetrix.com. For technical support outside the US, please visit our website for up-to-date contact information within your area.

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Related products

Proteinases

Product	Application	Pack size	Product number
Proteinase K	Protein degradation	100 mg 500 mg 2 x 500 mg	76230Y 76320Z 76230
Proteinase K Solution, 20 mg/ml	Protein degradation	100 mg	76225

DNA markers

Product	Application	Pack size	Product number
DNA Ladder, 1 kb Plus	Size determination of DNA	500 µl	76714
PCR Markers, 50-2000 bp	Size determination of DNA	250 µl	76710

Electrophoresis reagents

Product	Application	Pack size	Product number
Agarose, Hi-Res™, Separation, ≤1000 bp, Ultrapure	Gel electrophoresis	25 gm 100 gm 500 gm	10132
Agarose, LE, Ultrapure	Gel electrophoresis	25 gm 100 gm 250 gm 500 gm 1 kg	32802
DNA Loading Buffer, (OXG), 6X	Gel electrophoresis	1 ml 5 ml	76715
Sodium Dodecyl Solution (SDS), 10% Solution, Ultrapure	Gel electrophoresis	100 ml 500 ml 1 L	77504
TAE Buffer 10X Solution, Ultrapure	Gel electrophoresis	1 L 5 L	75904
TAE Buffer 50X Solution, Ultrapure	Gel electrophoresis	100 ml	74015
TBE Buffer 5X Solution, Ultrapure	Gel electrophoresis	1 L 5 L	75891
TBE Buffer, 10X, Ready-Mixed Powder, Ultrapure	Gel electrophoresis	6 x 200 ml	70454
Water, Nuclease-Free, Ultrapure	Gel electrophoresis	10 x 1 ml 100 ml 500 ml 1 L 5 L	71786

Ultrapure nucleotides

Product	Application	Pack size	Product number
PCR Nucleotide Mix, 10 mM each of dATP, dCTP, dGTP, and dTTP	RT and/or PCR, nucleotides	500 µl 2 x 500 µl	77212
PCR Nucleotide Mix, 25 mM each of dATP, dCTP, dGTP, and dTTP	RT and/or PCR, nucleotides	500 µl	77119

Additives for PCR

Product	Application	Pack size	Product number
Betaine, 5 M Solution, Ultrapure	Supplement for RT and/or PCR	1.5 ml 5 x 1.5 ml 10 ml	77507