

life

Dynabeads® DNA DIRECT™ Blood

For the isolation of PCR-ready
genomic DNA from blood

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Kit Contents and Storage

Storage

Upon receipt, store all components of the Dynabeads® DNA DIRECT™ Blood kit at 2°C to 8°C. **Do not freeze.**

All components are guaranteed stable until the expiration date printed on the label when stored unopened at 2°C to 8°C. Store the vial containing Dynabeads® in lysis buffer (vial 2) upright to keep the beads in solution, as drying of the Dynabeads® may result in reduced performance. Resuspend Dynabeads® fully prior to use.

Kit contents

The components included in the Dynabeads® DNA DIRECT™ Blood kit are listed in the following table.

Sufficient reagents are provided in the kit to perform 100 isolations. All components are quality controlled and tested to be free of contaminating DNA.

Component	Composition	Volume
Red Cell Lysis Buffer (supplied as a concentrate)	1.6 M sucrose 5% Triton® X-100 25 mM MgCl ₂ 60 mM Tris-HCl pH 7.5	30 mL
Dynabeads® DNA DIRECT™ Blood	Contains 1–5% NaOH	20 mL
Washing Buffer, 10X	0.4 M NaCl	30 mL
Resuspension Buffer, 1X	10 mM Tris-HCl pH 8	30 mL

Product use

For Research Use Only. Not for human or animal therapeutic or diagnostic use.

Description of the System

About the Kit

System overview

Dynabeads® DNA DIRECT™ Blood is designed for the simple and rapid isolation of high quality PCR-ready genomic DNA from blood. This cost-effective and efficient procedure is completed in a single tube in only 15 minutes.

DNA isolation involves cell lysis to release DNA and then adsorption of the DNA to the surface of the Dynabeads®. This is followed by magnetic separation of the intact DNA/Dynabeads® complex and subsequent washing to remove residual contaminants and potential PCR-inhibitors from the isolated DNA.

After resuspension, the complex is ready for direct use in downstream PCR-reactions. The DNA may be eluted from the Dynabeads® by a short incubation at 65°C.

Dynabeads® DNA DIRECT™ Blood is ideal for DNA isolation from multiple samples and multiple PCR-analyses on each sample. The method can be automated.

Note

To isolate PCR-ready genomic DNA from small quantities of a wide variety of crude sample materials, use Dynabeads® DNA DIRECT™ Universal. See **Accessory Products** (page 21) for ordering information.

Continued on next page

About the Kit, continued

Product Performance

- Dynabeads® DNA DIRECT™ Blood allows reproducible isolation of genomic DNA as determined by PCR. The amount of genomic DNA isolated depends on the number of nucleated cells present in the sample. From 100 µL blood the yield is typically 1–5 µg DNA. More DNA can be obtained with buffy coat as the starting sample.
- Isolated DNA is free of RNA and contaminants that may inhibit PCR. The DNA is of high integrity and high molecular weight. PCR-results show excellent reproducibility and high sensitivity.
- The procedure is simple and can be completed in as little as 15 minutes. Multiple samples may be handled in parallel and the isolation process can be automated.
- Genomic DNA isolated using Dynabeads® DNA DIRECT™ Blood has been successfully used as template in PCR from the following (see **Sample Preparation** page 8):
 - Fresh capillary blood
 - EDTA-, citrate-, ACD-, and heparin-anticoagulated blood
 - Blood stored at 2°C to 8°C or room temperature for up to one week
 - Frozen blood
 - Buffy coat
 - Bone marrow

Continued on next page

About the Kit, continued

Product Performance, continued

- Dynabeads® DNA DIRECT™ Blood has been successfully tested on blood samples from individuals with white blood cell counts below the normal adult human range (tested on $3.2\text{--}8.1 \times 10^6$ cells/mL, normal range is $4.7\text{--}7.3 \times 10^6$ cells/mL).
 - For samples containing a low white blood cell count, a higher yield and better reproducibility has been observed both for genomic DNA isolation and PCR-amplification.
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System specifications

- The kit supports 100 isolations from 100 μL of blood (see **Standard Protocol** page 9).
 - Each unit (200 μL) of Dynabeads® isolates enough high quality genomic DNA for at least 100 PCR-amplifications per isolation.
 - The protocol can be modified to allow isolation from 500 μL blood samples (see **Large Scale Protocol** page 14), providing enough template-DNA for 1000 PCR-amplifications.
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Methods

General Guidelines

Handling the DNA/ Dynabeads® Complex

- Add Dynabeads® to the sample in a single rapid pipetting action, a gelatinous complex of DNA and Dynabeads® forms. Do not mix further, as this damages the DNA/Dynabeads® complex and reduces the yield of DNA isolated.
- To avoid loss of material, do not shear the complex until after the Resuspension Buffer is added. To avoid drawing the complex into the pipette tip when aspirating the supernatant we recommend stepwise removal of the supernatant.
- Wash thoroughly to remove contaminants. Add Washing Buffer into the microcentrifuge tube in a single, rapid pipetting action so that the DNA/Dynabeads® complex is swirled around in the buffer. The addition of Washing Buffer washes the DNA/Dynabeads® complex off the tube wall in one piece. Some uncomplexed particles may be seen due to excess binding capacity.
- Remove all Washing Buffer between each wash.
- After three washes, the DNA/Dynabeads® complex is disrupted by resuspending in Resuspension Buffer. Thoroughly resuspend by repeatedly pipetting the complex up and down until homogeneous in appearance. Do not stir using the pipette tip as DNA may stick to the tip. Avoid pipetting air into the homogenized complex, as this may cause foaming.

Continued on next page

General Guidelines, continued

Choice of Magnet and Tubes

DynMag™ magnets pull the DNA/Dynabeads® complex to the tube wall so the supernatant can be easily removed without disturbing the pellet.

By scaling down the procedure it is possible to isolate genomic DNA from small samples in a 96-well plate using the DynMag™ 96 Side Skirted or DynMag™ 96 Side (manually or automated).

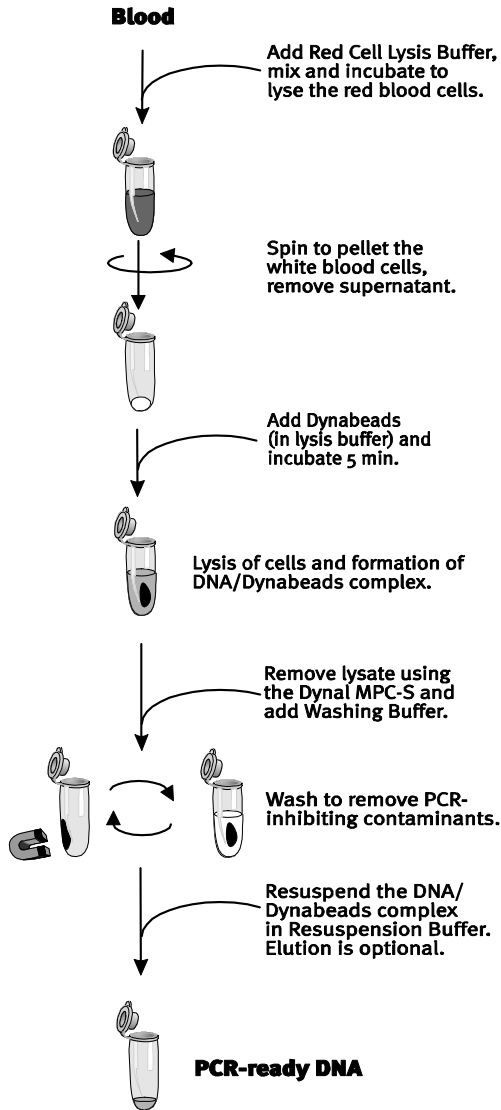
Note

Hypercellular samples (e.g., buffy coat, bone marrow and blood from individuals with acute infections) contain high numbers of nucleated cells and are thus richer in DNA than normocellular samples. This excess DNA may make the DNA/Dynabeads® complex harder to handle. In these cases, reduce the quantity of sample used in the procedure. Alternatively, increase the volume of Dynabeads®.

Experimental Overview

Workflow

The flow chart for purifying PCR-ready genomic DNA using the Dynabeads® DNA DIRECT™ Blood kit is shown in the following schematic.



Genomic DNA Purification Procedure

Introduction

The protocols included are for the isolation of genomic DNA from 100 μL of blood (**Standard Protocol**, page 9) or 500 μL of blood (**Large Scale Protocol**, page 14) in as little as 15 minutes.

Required materials

- DynaMag™ magnet: see www.lifetechnologies.com/magnets
 - Sterile, round-bottomed 2-mL microcentrifuge tubes
 - Micropipettors and sterile, disposable aerosol resistant pipette tips
 - 65°C water-bath or heat block (only needed for elution)
 - Phosphate Buffered Saline pH 7.4 (PBS) (for buffy coat sample preparation)
 - Sterile PCR-grade water for dilution of supplied buffers
 - Microcentrifuge
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Take care when handling and opening tubes of blood to avoid creating aerosols of potentially infectious material. See **Biological Hazard Safety** (page 23) for more information.

Dynabeads® DNA DIRECT beads as provided are in suspension containing 1–5% NaOH and should be handled with care.

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Genomic DNA Purification Procedure, continued

Sample Preparation

- Fresh capillary and anticoagulated blood (EDTA, ACD, citrate and heparin) may be used directly in the protocols provided.
 - Mix blood stored at room temperature or 2°C to 8°C on a vortex mixer for 2–3 seconds prior to aliquots being removed. Close the tube cap carefully before mixing. Take care when opening tubes to avoid creating aerosols of potentially infectious material.
 - Thaw frozen blood samples completely and mix on a vortex mixer for 2–3 seconds prior to aliquots being removed. Freezing lyses red blood cells, but still perform the Red Cell Lysis step to remove hemoglobin from the sample. Avoid repeated freeze/thaw cycles.
 - Buffy coat contains 2–4 times the number of white blood cells per volume compared to fresh blood. Only use 50 µL of buffy coat diluted with 50 µL PBS pH 7.4 as starting material for the standard protocol.
 - Bone marrow samples also contain a high amount of DNA. Use 10–20 µL bone marrow as starting material when following the standard protocol.
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Standard Protocol

Prepare Reagents

Important: Take care to avoid DNA and microbial contamination. Dispose of contaminated materials and decontaminate work surfaces correctly.

- Dynabeads® DNA DIRECT Blood is supplied ready-to-use in an aqueous lysis buffer containing salts, detergent and 1–5% sodium hydroxide. Resuspend Dynabeads® thoroughly by shaking gently to obtain a homogeneous dispersion of beads in solution.
- Dilute Red Cell Lysis Buffer 1:5 using sterile PCR-grade water and equipment before use. Keep on ice until use.

Important: The working concentration of the Red Cell Lysis Buffer is specific for each of the two different protocols described. See **Large Scale Protocol** (see page 14) for additional Red Cell Lysis Buffer dilution instructions.

Note: The exact volume supplied in the bottle may vary. Determine the actual volume if you intend to dilute the entire volume for use.

- Dilute Washing Buffer 1:10 using sterile PCR-grade water and equipment before use. The working concentration of the Washing Buffer is 1:10 for both protocols (referred to as 1X Washing Buffer).
- Bring the Dynabeads®, 1X Washing Buffer, and Resuspension Buffer to room temperature before use. Use Red Cell Lysis Buffer at 2°C to 8°C.

Continued on next page

Standard Protocol, continued

Cell Lysis

Read General Guidelines (see page 4) **before proceeding.**

1. In a 2-mL round-bottomed microcentrifuge tube, mix 100 μL anti-coagulated blood with 1 mL pre-diluted (1:5), chilled Red Cell Lysis Buffer.
Note: If less than 10 μL of blood is used as starting material, omit Red Cell Lysis step from the protocol.
2. Incubate at room temperature with occasional shaking of the tube until the red cells have lysed and the solution is clear.
Red Cell Lysis results in a clear solution of bright red color (lysis is usually complete within 5 minutes).
3. Centrifuge cells at $200 \times g$ for 30 seconds in a microcentrifuge.
A small, white or light grey hemoglobin-free pellet of cells should be visible. Remove and discard the supernatant.

Isolate DNA

1. Immediately add 200 μL (1 unit) of Dynabeads[®] to the cell pellet in a single rapid pipetting action to mix the components. **Do not** vortex or mix further.
2. Leave tube undisturbed at room temperature for 5 minutes.
3. Place tube on the magnet. Allow the DNA/Dynabeads[®] complex to move to the tube wall for 1 minute.
4. Carefully pipet off and discard the supernatant. The DNA/Dynabeads[®] complex will have a brown gelatinous appearance.
Important: During this step and subsequent washes, ensure that the complex is not disturbed or broken up.

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Standard Protocol, continued

Wash DNA/ Dynabead® Complexes

1. Remove the tube from the magnet.
2. Add 1 mL 1X Washing Buffer at room temperature to the tube in a single, rapid pipetting action to wash the DNA/Dynabeads® complex off the wall of the tube. Do not break up the complex as this will reduce DNA yield.
3. Replace tube on the magnet. Let the DNA/Dynabeads® complex move to the side of the tube and after 1 minute, or when supernatant has cleared, pipet off and discard the supernatant.
4. Repeat steps 1–3 twice, each time ensuring that the supernatant is completely removed and the DNA/Dynabeads® complex is intact.
5. Remove the tube from the magnet and thoroughly resuspend the DNA/Dynabeads® complex in 200 µL Resuspension Buffer or a volume suitable for your PCR application.

Note: This DNA/Dynabeads® suspension can be used directly in PCR-amplification reactions.

Note

Eluted/non-eluted DNA from 100 µL of blood is sufficient for at least 100 PCR-reactions.

If the DNA is to be stored at 2°C to 8°C or –20°C, elute the DNA as described in **Elute DNA** (see page 12) to prevent autocatalytic degradation.

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Standard Protocol, continued

Elute DNA

If required, elute the DNA.

1. Incubate the DNA/Dynabeads® suspension at 65°C for 5 minutes.
 2. Immediately place the tube in the magnet. Allow the DNA/Dynabeads® complex to move to the tube wall for 1 minute.
 3. Transfer the genomic DNA containing supernatant to a clean tube. Elution results in a clear, colorless, bead-free solution.
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DNA Quantitation

If determination of DNA concentration by (A_{260}/A_{280}) absorbance measurements is required, elute the DNA off the beads. Ensure that there are no beads left in the solution as they will negatively interfere with the spectrophotometric readings.

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Standard Protocol, continued

PCR-Amplifications

- Homogenized DNA/Dynabeads® in Resuspension Buffer can be used directly for PCR as the presence of Dynabeads® will not have any adverse effect. If the DNA is to be stored prior to PCR-amplification, elution is recommended.
 - Isolated DNA is denatured (due to the presence of NaOH in the lysis buffer) and available for PCR-amplifications. To increase the specificity of primer annealing to the denatured DNA, do not place the PCR reaction-mixture on the thermal cycler until the temperature is well above the primer annealing temperature or perform “hot start” PCR.
 - Use 1% of the homogenized DNA/Dynabeads® complex in Resuspension Buffer as DNA-template for one 30–35 cycle PCR-reaction. Up to a maximum of 5% of the complex or up to 50% of the eluted DNA can be used as starting material for PCR (50 µL reaction volume).
Note: For PCR-based tissue typing this may be less template than is generally recommended. Due to the denatured state of the isolated DNA it will be more available for PCR amplifications.
 - Use PCR-profiles with 30-35 cycles.
 - Some PCR-reactions are very sensitive to the amount of template-DNA used. In such cases, perform a titration of the DNA/Dynabeads® complex and compare eluted with non-eluted template DNA.
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Large Scale Protocol

Prepare Reagents

Initially use the **Standard Protocol** (page 9) in its 100- μ L format to become familiar with the procedure before scaling up. Use 2-mL round-bottomed microcentrifuge vials when working with 500 μ L of blood due to the size of the white blood cell pellet after centrifugation and the handling of the DNA/Dynabeads[®] complex.

Important: In this specific protocol the Red Cell Lysis Buffer is used in two versions.

- Dilute Red Cell Lysis Buffer using sterile PCR-grade water and equipment as follows:

Red Cell Lysis Buffer B1:

5 mL concentrate + 44 mL PCR-grade water + 1 mL 0.5 M EDTA pH 8

Red Cell Lysis Buffer B2:

5 mL concentrate + 45 mL PCR-grade water

Keep on ice until use.

Note: The exact volume supplied in the bottle may vary. Determine the actual volume if you intend to dilute the entire volume for use.

- Resuspend Dynabeads[®] thoroughly by shaking gently to obtain a homogeneous dispersion of beads in solution.
- Dilute Washing Buffer 1:10 using sterile PCR-grade water and equipment before use.
- Bring the Dynabeads[®], 1X Washing Buffer, and Resuspension Buffer to room temperature before use. Use Red Cell Lysis Buffer at 2°C to 8°C.

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Large Scale Protocol, continued

Cell Lysis

Read General Guidelines (see page 4) before proceeding.

1. Add 0.5 mL anti-coagulated whole blood to a 2-mL round-bottomed microcentrifuge tube.
2. Add 1 mL Red Cell Lysis Buffer B1, cap tightly and mix by inverting the tube several times until the red cells have lysed and the solution is clear.
3. Centrifuge at $200 \times g$ for 10–30 seconds to pellet white blood cells. Remove and discard the supernatant.
4. Disperse the cell pellet by flicking the tube several times or briefly vortexing.
5. Add 1 mL Red Cell Lysis Buffer B2, cap securely, and mix by inverting. If lumps are observed, leave to settle for one minute and transfer supernatant to a new tube.
6. Centrifuge cell lysate at $200 \times g$ for 30 seconds in a microcentrifuge. Remove and discard the supernatant.
A small, white or light grey, hemoglobin-free pellet of cells should be visible.

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Large Scale Protocol, continued

Isolate DNA

1. Vortex or flick the tube to break up the cell pellet and add 200 μL (1 unit) of Dynabeads[®] in a single rapid pipetting motion.
 2. Immediately transfer the contents of the tube (using the same pipette tip) to a clean 2-mL round-bottomed microcentrifuge tube. No incubation is necessary, but for maximum yield incubate at room temperature for 5 minutes. Do not vortex or mix further.
At this point a complex of DNA/Dynabeads[®] with a brown, gelatinous appearance is visible.
 3. Place the tube in the magnet for 1 minute and allow the DNA/Dynabeads[®] to collect on the tube wall.
 4. Carefully aspirate and discard the supernatant.
Important: During this step and subsequent washes, ensure that the complex is not disturbed or broken up.
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Note

If samples from patients with abnormal white cell counts are used, the DNA/Dynabeads[®] complex might not form or be too large to handle. In these cases, adjust the initial volume of blood used accordingly. See **Troubleshooting**, page 19.

Continued on next page

Large Scale Protocol, continued

Wash DNA/ Dynabead® Complexes

1. Remove the tube from the magnet.
2. Add 1 mL 1X Washing Buffer at room temperature to the tube in a single, rapid pipetting action to flush the DNA/Dynabeads® complex from the tube wall. Do not break up the complex.
3. Replace the tube on the magnet for 1 minute and allow the DNA/Dynabeads® complex to move to the tube wall. Carefully aspirate and discard the supernatant.
4. Wash the complex twice more by repeating steps 1–3 ensuring the supernatant is completely removed and the complex remains intact.
5. Remove the tube from the magnet. Add 200 µL (or a volume suitable for your PCR application) of Resuspension Buffer and homogenize the complex by repeatedly pipetting up and down.

Note: This homogeneous DNA/Dynabeads® suspension can be used directly for PCR-amplification. If the DNA is to be stored at 2°C to 8°C or -20°C elute the DNA as described in **Elute DNA** (page 18), to prevent autocatalytic degradation.

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Large Scale Protocol, continued

Elute DNA

If required, elute the DNA.

1. Incubate the DNA/Dynabeads® suspension at 65°C for 5 minutes.
 2. Immediately place the tube in the magnet. Allow the DNA/Dynabeads® complex to move to the tube wall for 1 minute.
 3. Transfer the genomic DNA containing supernatant to a clean tube. Elution results in a clear, colorless, bead-free solution.
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DNA Quantitation

If determination of DNA concentration by (A_{260}/A_{280}) absorbance measurements is required, elute the DNA off the beads. Ensure that there are no beads left in the solution as they will negatively interfere with the spectrophotometric readings.

PCR Amplification

Use 10–20 μL of the isolated and eluted DNA to prepare a working dilution sufficient for 100 PCR reactions. See **Standard Protocol, PCR-Amplifications** on page 13 for additional PCR guidelines. Store remaining DNA frozen at -20°C .

Troubleshooting

Observation	Cause	Solution
DNA/Dynabeads® complex does not form, or is small and fragmented	Very little DNA present	<ul style="list-style-type: none"> • Check the white blood cell count. • Increase sample quality and quantity.
DNA/Dynabeads® complex is unusually large and difficult to handle	Large amount of DNA present	<ul style="list-style-type: none"> • Refer to Handling the DNA/Dynabeads® Complex on page 4. • If problem persists, use less sample or more Dynabeads®.
DNA/Dynabeads® complex is difficult to break up at the resuspension step	Insufficient pipetting	<ul style="list-style-type: none"> • Continue pipetting longer. • Move the tube rapidly over an uneven surface, e.g., iron thread tube rack, to achieve a shaking (not vortexing) motion.
	Too large aperture on pipette tip	Use a tip with a smaller aperture.
PCR-background is high	Template-DNA or PCR-primers concentration may be too high	<ul style="list-style-type: none"> • Place the PCR reaction-mixtures on the thermal cycler when the temperature is 72°C or perform “hot-start” PCR. • Reduce the amount of template-DNA, PCR-primers, Mg²⁺ or enzyme used. • Elute the DNA prior to PCR-amplification.
	The isolated DNA contains contaminants	<ul style="list-style-type: none"> • Red Cell Lysis was not complete. Increase the incubation time or repeat the Red Cell Lysis step. • Add Washing Buffer with increased force. • Remove the supernatant completely at each washing step.

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Troubleshooting, continued

Observation	Cause	Solution
<p>PCR-amplification is observed using 5% of the isolated DNA, but not when using 1%</p>	<p>Very little DNA present</p>	<ul style="list-style-type: none"> • If fragmentation of the complex was observed during the washing steps, reduce the force used to add wash buffer. • Elution, if performed, may have been inefficient. Ensure that all Washing Buffer is removed prior to addition of Resuspension Buffer. Increase the force used to homogenize the complex at the resuspension step. • Increase sample quality and quantity.
<p>No PCR-amplification is observed when using 1–5% of the isolated DNA</p>	<p>Indicates the presence of PCR-inhibitors</p>	<ul style="list-style-type: none"> • Red Cell Lysis incomplete. Increase the incubation time or repeat the Red Cell Lysis step. • Add Washing Buffer more vigorously. • Ensure that the supernatant is completely removed at each washing step. • Use less starting material for the PCR.

Appendix

Accessory Products

Related Products

The following products may be used with the Dynabeads® DNA DIRECT™ Blood kit. For details, visit www.lifetechnologies.com or contact **Technical Support** (see page 22).

Product	Quantity	Cat. no.
DynaMag™-2	Each	12321D
SampleRack (for DynaMag™-2)	Each	12322D
DynaMag™ 96 Side Skirted	Each	12027
DynaMag™ 96 Side	Each	12331D
Dynabeads® DNA DIRECT™ Universal	300 isolations	63006
Dynabeads® Silane genomic DNA	96 isolations	37012D
Platinum® <i>Taq</i> DNA Polymerase High Fidelity	100 reactions	11304-011
Platinum® <i>Taq</i> DNA Polymerase	100 reactions	10966-018
UltraPure™ DNase/RNase-free Distilled Water	500 mL	10977-015
100 bp DNA Ladder	50 µg	15628-019

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@lifetechnologies.com**)
 - Search for user documents, Safety Data Sheets (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
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Safety Data Sheets (SDS)

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

Certificate of Analysis

The Certificate of Analysis is available at **www.lifetechnologies.com/support**. 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**.

Biological Hazard Safety



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/

Purchaser Notification

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General Information

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