

life

Dynabeads® DNA DIRECT™ Universal

For the isolation of PCR-ready genomic DNA
from small samples

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Kit Contents and Storage

Shipping and Storage

All components of the Dynabeads® DNA DIRECT™ Universal kit are shipped at ambient temperature.

Upon receipt, store all components at 2°C to 8°C. Freezing of the kit is not recommended.

Precautions should be taken to ensure that DNA or microbial contamination of the kit components does not occur. Ensure proper disposal of contaminated materials and decontamination of work surfaces.

The buffers and components provided with Dynabeads® DNA DIRECT™ Universal should be brought to room temperature and the Dynabeads® fully resuspended prior to use.

Important

Store vials containing Dynabeads® upright to ensure that the beads are covered with buffer. Drying of Dynabeads® may reduce their efficiency. If Dynabeads® become dried; resuspend the beads by keeping the vial in motion on a roller for up to 12 hours. This should restore the functionality of the Dynabeads®.

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Kit Contents and Storage, continued

Kit Contents The components included in the Dynabeads® DNA DIRECT™ Universal Kit are listed in the following table. Dynabeads® DNA DIRECT™ Universal is supplied with the necessary buffers and solutions, supporting DNA-isolation from 300 samples.

Component	Composition	Volume
Dynabeads® DNA DIRECT™ Universal (supplied ready-to-use in a lysis buffer)	—	60 mL
10X Washing Buffer	100 mM Tris-HCl, pH 7.5 1.5 M LiCl 1 mM EDTA	30 mL
Resuspension Buffer	10 mM Tris-HCl, pH 8	30 mL
10 M NaOH	Sodium Hydroxide solution, 40% (w/v)	5 mL



- The Dynabeads® suspension is corrosive. NaOH can cause burns of eyes and skin. May cause coughing, difficulty with breathing, lung damage, diarrhea, or shock if inhaled or ingested. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention.
 - This product contains 0.02% sodium azide as a preservative, which is cytotoxic. **Avoid pipetting by mouth!** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. When disposing through plumbing drains, flush with large volumes of water to prevent azide build up.
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Product Use **For Research Use Only.** Not for human or animal therapeutic or diagnostic use.

Description of the System

About the Kit

Product Description

Dynabeads® DNA DIRECT™ Universal is ready-to-use for the simple and rapid isolation of PCR-ready genomic DNA from small quantities of a variety of crude sample materials (e.g., clinical specimens, tissues from various species, cultured cells, and blood).

Dynabeads® are uniform, superparamagnetic, monodisperse polymer particles. The uniformity of both particle size and shape allows for the rapid and efficient binding of the target to the beads.

Dynabeads® DNA DIRECT™ Universal is supplied complete, with Dynabeads® (supplied in lysis Buffer), Washing Buffer and Resuspension Buffer.

System Overview

The process of DNA isolation relies upon cell lysis and subsequent adsorption of the released DNA to the surface of the Dynabeads® during a brief incubation.

The DNA/Dynabeads® complex is pulled to the side wall of the well by applying a magnetic field (DynaMag™ magnet or similar, see **Accessory Products** page 25). Magnetic separation of the intact DNA/Dynabeads® complex is followed by removal of the supernatant and subsequent washing to remove any residual contaminants and potential PCR inhibitors.

Finally, the complex is simply resuspended for direct use in downstream PCR reactions. Alternatively, the DNA may be eluted from the Dynabeads® with a short incubation at 65°C.

Continued on next page

About the Kit, continued

Advantages

The major advantages of using Dynabeads® DNA DIRECT™ Universal for isolation of PCR-ready DNA include:

- Cost-effective, simple, and reliable procedure is completed in a single tube in only 10 min.
- PCR results show excellent reproducibility in combination with a high level of sensitivity.
- Isolated DNA is of high integrity and high molecular weight (1).
- The sensitivity and efficiency of DNA capture enables successful isolation, even when the quantities of starting material are limited.
- Dynabeads® biomagnetic separation technology overcomes the need for time-consuming centrifugation steps and also avoids the use of hazardous chemicals that may require costly disposal.
- Dynabeads® DNA DIRECT™ Universal is equally suited to laboratories handling small, precious samples and those with high-throughput requirements.
- The method enables samples to be batched, so that a large number of samples can be processed simultaneously.
- Automation of the protocol is possible. Using Dynabeads® DNA DIRECT™ Universal and the automated protocol on a Tecan Genesis® RSP (Tecan AG, Switzerland), 48 samples can be processed in 60 min.

Continued on next page

About the Kit, continued

Sample Sources

Using Dynabeads® DNA DIRECT™ Universal, PCR-ready DNA has been successfully and reproducibly prepared directly from various solid tissues and cells including (see **References**):

- Cultured cells (3)
- Mouth wash (7)
- Buccal scrapes and sputum (6)
- Urine (7)
- Bile (7)
- Feces (7, 8)
- Cerebrospinal fluid
- Bone marrow (3, 7, 16, 17)
- Buffy coat
- Frozen blood
- Fresh capillary and anticoagulated blood
- Bacteria (1, 9–14)
- Algae (10)
- Fungi (10, 15)
- Plants (10)
- Mouse tails (6)
- Various other vertebrate tissue (3, 10, 18, 19)

Expected Results

A minimum quantity of mammalian sample will typically yield sufficient template for 10 PCR reactions. By optimizing sample quantities and lysis conditions, one unit (200 µL) Dynabeads® isolates at least 200 ng high quality genomic DNA to be used as template DNA for subsequent PCR amplification.

Continued on next page

About the Kit, continued

Automated Isolation of DNA from Blood Samples

Dynabeads® DNA DIRECT™ Universal is the preferred kit for the automated isolation of genomic DNA from blood. Small sample volumes ($\leq 30 \mu\text{L}$) makes red cell lysis and hemoglobin removal unnecessary. Buccal scrape samples have also shown good results. For more information, see **Sample Pre-treatment** page 9 (describing the use of Dynabeads® DNA DIRECT™ Universal for manual DNA isolation).

Dynabeads® DNA DIRECT™ Universal can be used with:

- anti-coagulated blood (using anti-coagulants such as ACD, citrate or EDTA)
- blood stored at room temperature or at 2°C to 8°C for up to one week
- frozen blood
- buffy coat

It is possible to use sample volumes of up to $30 \mu\text{L}$ human blood.

The yield of genomic DNA isolated using Dynabeads® DNA DIRECT™ Universal depends on the number of nucleated cells present in the sample as well as the species. Dynabeads® DNA DIRECT™ Universal has been successfully tested on blood samples from individuals with various white blood cell counts and also on blood from other mammalian sources. Blood from other mammalian sources may vary in white blood cell content and sample size should be adjusted not to exceed $1 \mu\text{g}$ DNA per sample.

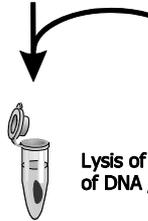
Experimental Overview

Purifying DNA Workflow

The following illustration shows the flow chart for purifying PCR-ready genomic DNA using the Dynabeads® DNA DIRECT™ Universal Kit.

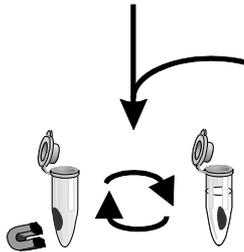
Starting sample

Add 1 unit (200 µL) fully resuspended Dynabeads® DNA DIRECT™ Universal in a single, rapid pipetting action and incubate for 5 min. at room temperature. Do not mix further.



Lysis of sample and formation of DNA / Dynabeads complex

Using the magnet remove the lysate and add 200 µL Washing Buffer. Do not break up the complex.



Wash twice using the magnet to remove PCR-inhibiting contaminants. Ensure that all Washing Buffer is removed between each wash.

Resuspend the DNA/Dynabeads complex in 20–40 µL Resuspension Buffer. Elution is optional.



PCR-ready DNA

Methods

General Guidelines

Handling the DNA/ Dynabeads® Complex

- The DNA/Dynabeads® complex forms after the Dynabeads® are added to the sample in a single, rapid pipetting action. The complex appears gelatinous.
- Do not vortex, pipet or mix further as shear forces may damage the complex and result in decreased yield.
- To avoid loss of material, it is important that the complex is kept intact until the Resuspension Buffer (or low-ionic strength buffer of choice) is added.
- Avoid drawing the DNA/Dynabeads® complex into the pipette tip when removing supernatant.
- Removing the supernatant in small aliquots reduces the likelihood of accidentally drawing the complex into a pipette tip.
- Although it is important that the DNA/Dynabeads® complex remains intact, the washing steps must be thorough enough to ensure removal of contaminants.
- Washing Buffer should be added to the tube in a single, rapid pipetting action, dislodging the DNA/Dynabeads® complex from the well wall in one piece. This will swirl the complex around in the buffer without requiring any further stirring or mixing action.
- Non-complexed Dynabeads® may be observed due to excess binding capacity.

Continued on next page

General Guidelines, continued

Handling the DNA/ Dynabeads® Complex, continued

- After the washes, the DNA/Dynabeads® complex is broken up in Resuspension Buffer, water or low-ionic strength buffer (e.g., TE).
 - Triturate the washed DNA/Dynabeads® complex with repeated pipetting in Resuspension Buffer until the suspension appears homogeneous.
 - Minimizing pipetting at this step results in genomic DNA of higher average molecular weight, but high molecular weight DNA takes a very long time to dissolve properly.
 - Do not stir the suspension with the pipette tip, as DNA may become stuck to the tip.
 - Avoid pipetting air into the suspension.
 - Resuspension is easier to perform if the complex is incubated in Resuspension Buffer, water or low-ionic strength buffer for a while prior to resuspension (it may be left at 2°C to 8°C over night). Using a pre-heated buffer can also facilitate resuspension.
 - Elution, if performed, should give a clear, colorless solution free of Dynabeads®.
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Sample Pre-treatment

Mouth Washes

Extract Mouth wash specimens as soon as possible after collection, although they may be stored at 4°C overnight.

1. Collect the sample by rinsing the mouth with 25 mL water.
2. Centrifuge the expelled sample at $650 \times g$ for 20 min, discard the supernatant.
3. Gently resuspend the cells in 10 mL sterile Phosphate Buffered Saline (PBS).
4. Centrifuge at $650 \times g$ for 10 min and discard all but 1 mL of the supernatant.
5. Gently resuspend the pellet in the remaining supernatant and transfer to a 1.5-mL microcentrifuge tube.
6. Centrifuge briefly at $650 \times g$ to form a pellet and discard the supernatant.
7. Estimate the size of the pellet and resuspend in an equal volume of molecular biology grade water (specimens may be stored for several days at -20°C after being resuspended in water).
8. Use 10 μL of the cell suspension per isolation as described in **General Sample DNA Isolation Protocol** on page 15.

Note

Some mouth wash specimens may have a high bacterial content. Bacterial DNA is co-isolated with the genomic DNA. If inhibition of PCR for a human genomic target is observed, dilution of the DNA may be required.

Continued on next page

Sample Pre-treatment, continued

Buccal Scrapes

1. Collect buccal scrapes using a simple plastic scraper.
2. Place the scraper in a microcentrifuge tube with 50 μ L PBS and spin down the sample.
3. Remove the PBS and the scraper and follow the **General Sample DNA Isolation Protocol** on page 15 using 40 μ L for total sample size.

Note: Dynabeads[®] DNA DIRECT[™] Universal is not suited for use with buccal swab samples.

Bile

1. Centrifuge a large volume of bile at 650 \times g for 10 min and discard the supernatant.
2. Resuspend the pellet in 1.5 mL sterile PBS and transfer the suspension to a 1.5-mL tube.
3. Centrifuge at 650 \times g for 10 min and discard the supernatant.
4. Estimate the size of the pellet and resuspend in an equal volume of molecular biology grade water.
5. Use 10 μ L cell suspension per isolation as described in **General Sample DNA Isolation Protocol** on page 15.

Note: Bile samples taken at ERCP when a surgical stent is inserted are often full of debris such as uric crystals and feces. DNA cannot be successfully extracted from these samples.

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Sample Pre-treatment, continued

Urine

1. Collect urine specimens fresh and do not store for more than 1–2 hours at 4°C.
 2. Centrifuge the sample at $650 \times g$ for 10 min, discard the supernatant.
 3. Add 10 mL sterile PBS, gently resuspend the cells and centrifuge at $650 \times g$ for 10 min.
 4. For highly chromogenic specimens discard the supernatant and repeat step 3.
 5. Discard all but 1 mL of the supernatant, gently resuspend the remaining pellet and transfer the suspension to a 1.5-mL microcentrifuge tube.
 6. Centrifuge briefly at $650 \times g$ to form a pellet and discard the supernatant.
 7. Estimate the size of the pellet and resuspend in an equal volume of molecular biology grade water (specimens may be stored for several days at -20°C after being resuspended in water).
 8. Use 10 μL of the cell suspension per isolation as described in **General Sample DNA Isolation Protocol** on page 15.
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Feces

1. Suspend 100 mg fecal sample in 300 μL buffer (500 mM Tris, 16 mM EDTA, 10 mM NaCl pH 9.0)
 2. Centrifuge at $10,000 \times g$ in a microcentrifuge for 2 min to pellet the fecal solids.
 3. Avoid disturbing the pale-colored surface layer over the solids and carefully remove and discard the supernatant.
 4. Transfer this surface layer to a clean tube and isolate the DNA as described in **General Sample DNA Isolation Protocol** on page 15.
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Sample Pre-treatment, continued

Fungi

- Grind fruit-bodies (1–3 mg air-dried or 3–20 mg fresh) with a forceps for about 2 min to mechanically break open the hard cell walls and thereby increase DNA yield.
 - The highest yields have been obtained from dried fruit-bodies.
 - Incubation with Dynabeads® at 65°C for 15 min prior to DNA isolation can give increased DNA yields.
 - Mycelium is isolated by scraping the surface of mycelium grown on agar plates with a spatula.
 - About 1 mg wet weight commercial bakers yeast (*S. cerevisiae*) should be used for each DNA isolation.
 - Mechanical, chemical or enzymatic digestion of the yeast cell walls should increase the yield of genomic DNA.
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Plants

Approximately 30–100 mg fresh plant leaf is used per isolation.

1. Homogenize the plant leaf for 2 min in liquid nitrogen with a pestle to mechanically break open the hard cell walls and thereby increase DNA yield.
2. Incubation with Dynabeads® at 65°C for 15 min prior to DNA isolation can give increased DNA yields.

Both nuclear and chloroplast DNA from *Arabidopsis thaliana* (dicot) and barley (*Hordeum vulgare*, monocot) have been isolated.

Algae

Collect approximately 5×10^5 cells by centrifugation at $7500 \times g$ for 5 min.

Both genomic and chloroplast DNA from *dinophyceae* and genomic DNA from *chlorophyceae*, *phaeophyceae*, *crysophyceae* and *rhodophyceae* have been isolated.

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Sample Pre-treatment, continued

Bacteria

1. Pellet $\sim 0.2\text{--}2 \times 10^8$ cells from culture in a microcentrifuge at $15,000 \times g$ for 5 min.
2. Incubation with Dynabeads® at 65°C for 15 min prior to DNA isolation gives a substantial increase in DNA yield for some cyanobacteria.

Note: Gram-positive and Gram-negative bacteria, as well as cyanobacteria, have been tested.

Cultured Cells

- Cultured cells can be used directly, but may also be washed twice in DNA-free PBS (pH 7.2–7.6).
- Resuspend cultured cells at 1×10^6 cells/mL.
- We recommend using a maximum of 10 μL of the cell suspension per isolation.

Note: Proliferating cultured cells may contain more DNA than quiescent white blood cells, see **Blood Samples**.

Vertebrate

About 50–100 mg fish fins (epithelium), 10–50 mg mammalian liver or muscle tissue conserved in 96% ethanol have been used directly for DNA isolation (see **General Sample DNA Isolation Protocol** on page 15) and PCR amplification of mitochondrial DNA (D-loop) from perch, lynx, and arctic fox.

Mouse Tail

Place 3–5 mg mouse tail (~ 1 mm tail section) into a 1.5-mL microcentrifuge tube. Squeeze the tail section firmly against the wall of the microcentrifuge tube to disrupt the outer cell layers. Following the addition and incubation with the Dynabeads® in lysis buffer shake off any Dynabeads® clinging to the tail remnants. The tail remnants should be removed after the incubation period (**General Sample DNA Isolation Protocol** step 2, page 15.).

DNA Isolation Protocol

Introduction

The protocol is for the direct manual isolation of genomic DNA from a variety of crude sample materials (e.g., clinical specimens, tissues from various species, cultured cells) using Dynabeads® DNA DIRECT™ Universal.

The protocol can be modified for the automated isolation of PCR-ready DNA from blood, buccal scrapes and other sample types (see **96-Well Protocol for Blood Samples**, page 17).

Required Materials

Components required but not supplied:

- Micropipettors
- Sterile, disposable pipette tips (aerosol resistant)
- Sterile 1.5-mL microcentrifuge tubes
- DynaMag™-2 is recommended (Cat. no. A12321D).
- Phosphate Buffered Saline (PBS) pH 7.2–7.6
- Water bath or heating block (for optional elution).
- Microcentrifuge capable of achieving $>10,000 \times g$

Components supplied with the kit:

- Dynabeads® DNA DIRECT™ Universal
 - Washing Buffer,
 - Resuspension Buffer
 - 10 M NaOH 40 % (w/v)
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DNA Isolation Protocol, continued

Before Starting

- Equilibrate the buffers provided with Dynabeads® DNA DIRECT™ Universal to room temperature prior to use.
 - Dilute the supplied 10X Washing Buffer to 1X concentration using sterile and PCR-grade water and equipment before proceeding with DNA isolations.
 - During storage, the Dynabeads® settle to the bottom of the bottle. Prior to use, thoroughly resuspend the Dynabeads® by gently shaking the bottle to obtain a homogeneous dispersion of Dynabeads® in solution. Avoid foaming. **Do not vortex.**
 - The detergent in the lysis buffer may precipitate when stored at 4°C. If this should happen, mix and slightly heat the bottle to resuspend, obtaining a homogeneous dispersion of beads in solution. Avoid foaming. **Do not vortex.**
Note: Chaotropes like GTC may also cause precipitation of the detergent in the lysis buffer.
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For Best Results

- Adjust your sample size (determined also by the degree of lysis) to match the Dynabeads® capacity. Lysis of the sample should release 200–500 ng DNA (for sample preparation, see **Sample Pre-treatment** on pages 8–12).
 - Avoid high concentrations of salt in your sample.
 - Avoid chaotropic salts like GTC.
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DNA Isolation Protocol, continued

General Sample DNA Isolation Protocol

1. Place your sample (prepared as described in **Sample Pre-treatment** on pages 8–12) in a 1.5-mL microcentrifuge tube and add 200 μ L (1 unit) fully resuspended Dynabeads[®] (supplied in lysis buffer) in a single rapid pipetting action.
The swirling mixes the components sufficiently. Do not vortex or mix further. A few seconds after the addition of the Dynabeads[®], a DNA/Dynabeads[®] complex should be visible, unless your sample has a color that masks this.
2. Leave the tube at room temperature for 5 min. Continuous agitation is not required.
3. Place the tube in a magnet for 2 min, carefully remove and discard the supernatant.
The DNA/Dynabeads[®] complex has a dark brown gelatinous appearance.
4. Remove the tube from the magnet. Add 200 μ L 1X Washing Buffer in a single rapid pipetting action so that the complex is flushed off the side wall. Do not mix further.
5. Place the tube in the magnet and leave for 30 seconds or until the supernatant has cleared. Carefully remove and discard the supernatant.
6. Repeat steps 4 and 5 once.
If a large proportion of the isolated DNA is being used for one PCR reaction repeat steps 4 and 5 twice (see **PCR Amplifications** on page 22). The DNA/Dynabeads[®] complex becomes more compact during the washing steps.
7. Remove the tube from the magnet. Resuspend the DNA/Dynabeads[®] complex in 20–40 μ L Resuspension Buffer (alternatively water or low-ionic strength buffer of choice i.e., TE).

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DNA Isolation Protocol, continued

General Sample DNA Isolation Protocol, continued

8. Pipet the complex up and down 30–40 times or until the suspension is homogeneous.
9. If required, the DNA can be eluted off the Dynabeads® by incubation at 65°C for 5 min. Immediately place the tube in the magnet and leave for 30 seconds. Transfer the supernatant, containing DNA, to a new sterile tube.

Up to 10% of the DNA/Dynabeads® suspension from step 7 can be used as template for PCR without adverse effect due to the presence of the Dynabeads®. We recommend that not more than half of the eluted DNA after step 9 should be used for one PCR. For further information on PCR amplification see page 22.

Note

If storage of DNA is required at –20°C or for more than one week at 2°C to 8°C, elution of DNA (step 9) is recommended. Determination of DNA concentration by absorbance (A_{260}/A_{280}) measurement (2) requires elution of the DNA from the Dynabeads®.

Quantitation

DNA must first be eluted off the Dynabeads® for determination of DNA concentration by absorbance measurement (2). Ensure no Dynabeads® remain in the DNA solution as the beads interfere with the spectrophotometric readings. The DNA concentration can also be checked by agarose gel electrophoresis. If NaOH is used, the isolated DNA will be denatured (partially single stranded) and highly available for PCR-amplification.

Note: Single-stranded DNA has a much lower binding efficiency for ethidium bromide compared to double-stranded DNA.

96-Well Protocol for Blood Samples

Introduction

This protocol is for the automated isolation of genomic DNA from blood using Dynabeads® DNA DIRECT™ Universal.

The protocol can be modified for the automated isolation of PCR-ready DNA from buccal scrapes and other sample types.

Expected Results

One unit (200 µL) of Dynabeads® applied to 30 µL of blood isolates between 600 ng–1 µg of high quality genomic DNA. This amount of DNA is sufficient for 30–50 PCR amplifications, see **PCR Amplifications** page 22.

Required Materials

Additional components required for automated sample handling:

- Liquid-handling robot or eight channel (50–200 µL) pipette with appropriate tips.
 - The Te-MagS from Tecan or DynaMag™-96 Side Skirted magnet is recommended see Accessory Products, page 25.
 - 96-well skirted PCR-plates.
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96-Well Protocol for Blood Samples, continued

Before Starting

- Equilibrate the buffers provided with Dynabeads® DNA DIRECT™ Universal to room temperature prior to use.
- Dilute the supplied 10X Washing Buffer to 1X concentration using sterile and PCR-grade water and equipment before proceeding with DNA isolations.
- Resuspend Dynabeads® before use by gentle shaking to obtain a homogeneous dispersion of Dynabeads® in solution. Avoid foaming. **Do not vortex.**
- The detergent in the lysis buffer may precipitate when stored at 4°C. If this should happen, mix and slightly heat the bottle to resuspend, obtaining a homogeneous dispersion of beads in solution. Avoid foaming. **Do not vortex.**
Note: Chaotropes like GTC may also cause precipitation of the detergent in the lysis buffer.

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96-Well Protocol for Blood Samples, continued

Blood Sample Guidelines

- 5–30 μL fresh capillary and anticoagulated blood (EDTA/ACD/citrate) may be used directly in the protocol. (4, 5, 6, 20)
Note: Fresh blood coagulates within minutes if an anticoagulant is not added.
 - Heparin treated blood is not recommended. If used, limit the sample size to 5 μL with 200 μL Dynabeads[®].
 - Briefly mix stored or thawed blood on a vortex mixer prior to use.
 - Hypercellular samples contain high numbers of nucleated cells and are consequently richer in DNA. This excess DNA may make the DNA/Dynabeads[®] complex difficult to handle and the quantity of sample introduced into the procedure should be reduced (e.g., 2–3 μL buffy coat, 1–2 μL bone marrow). Alternatively, the quantity of Dynabeads[®] used may be increased.
 - With small blood samples (<20 μL) and buccal scrape samples, the number of washing steps may be reduced from 3 to 2. If NaOH is added (10 μL 10M NaOH per 200 μL Dynabeads[®] in lysis buffer), blood samples up to 30 μL can be used. The addition of NaOH makes the DNA/Dynabeads[®] complex easier to handle.
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Note

The volumes mentioned here are for human blood samples. Dynabeads[®] DNA DIRECT[™] has been successfully used for isolation of DNA from blood from other species (3, 10).



For the isolation of PCR-ready DNA from larger volumes (up to 500 μL) of whole blood, buffy coat and bone-marrow, Dynabeads[®] DNA DIRECT[™] Blood (see **Accessory Products** page 25) is recommended.

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96-Well Protocol for Blood Samples, continued

Blood Sample Isolation Protocol

1. Dispense your blood sample into individual wells of a skirted 96-well plate. For maximum DNA yield, use up to 30 μL human blood. For blood samples $\leq 10 \mu\text{L}$ proceed directly to step 3.
2. When $>10 \mu\text{L}$ blood is used, determine the total volume of Dynabeads[®] in lysis buffer required (i.e. 100 samples \times 170 μL Dynabeads[®] = 17 mL). Mix the lysis solution (containing the Dynabeads[®]) with 1/20 volume (17 mL/20 = 0.85 mL) 10 M NaOH prior to use.
Note: After adding NaOH, the Dynabeads[®] solution becomes cloudy.
CAUTION: The NaOH suspension is corrosive. Wear suitable gloves and eye/face protection.
3. Add 170 μL Dynabeads[®] resuspended in lysis buffer to sample wells in a single rapid pipetting action with enough force to ensure mixing. Do not mix further.
4. Leave at room temperature for 5 min. Avoid continuous agitation.
5. Place the 96-well plate on the DynaMag[™]-96 Side Skirted for 1–2 min.
6. Aspirate lysate without touching the DNA/Dynabeads[®] complex.
To achieve this it might be necessary to aspirate off center, along the wall opposite the complex. The DNA/Dynabeads[®] complex has a dark brown gelatinous appearance. During this step and subsequent washing steps, take care not to break up the complex.
7. Remove the 96-well plate from the magnet.
8. Add 200 μL 1X Washing Buffer to the wells. The Washing Buffer must be dispensed along the wall above the complex to ensure that the complex is flushed off the wall.

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96-Well Protocol for Blood Samples, continued

Blood Sample Isolation Protocol, continued

9. Place the 96-well plate back on the magnet for 1–2 min or until the supernatant has cleared.
10. Aspirate Washing Buffer without touching the DNA/Dynabeads® complex.
11. Repeat steps 7–10 once (<20 µL blood and buccal scrape samples) or twice (>20 µL blood samples).
12. Remove the 96-well plate from the magnet.
13. Add 100 µL Resuspension Buffer, water, or low-ionic strength buffer (TE or similar) to the wells. Dispense along the wall above the complex to ensure that the complex is flushed off the wall.
14. Resuspend the complex by pipetting up and down until suspension is fully homogenized.
The number of pipetting steps depends on the liquid handling robot and the sample volume used. Using a Biomek and 20–30 µL blood pipet the complex 80–100 times to ensure homogeneity.
15. If required, the DNA can be eluted off the Dynabeads® in Resuspension Buffer, water, or low-ionic strength buffer by incubation at 65°C for 5 min. The complex must be fully resuspended before elution: The elution buffer can be pre-heated to 65°C. After incubation, place tube in magnet stand for 30 seconds and transfer supernatant to a clean tube.

Note: Resuspension is more efficient if aspiration is performed at the center of the well and dispensing is performed off center than if aspiration and dispensing are performed at the same location.

Note: If storage of DNA is required at –20°C or for more than one week at 2°C to 8°C, elution of DNA (step 15) is recommended.

Note: Quantitation of DNA by absorbance (A_{260}/A_{280}) measurement (2) requires elution of the DNA from the Dynabeads® (see page 16).

PCR Amplifications

Template Considerations

- DNA to be used as a template for PCR amplification must be free of PCR-inhibiting contaminants. Dynabeads® DNA DIRECT™ Universal is designed for the direct isolation of PCR-ready genomic DNA of purity to meet this requirement.
 - The presence of Dynabeads® does not adversely affect the PCR reaction.
 - We recommend using no more than 10% of the resuspended DNA/Dynabeads® complex (in Resuspension Buffer, water or low-ionic strength buffer), or up to 50% of the eluted DNA as template for PCR (50 µL) amplifications.
 - When 200 µL Dynabeads® is applied to 30 µL of human blood, between 600 ng–1 µg of high quality genomic DNA will be isolated and can be used as template DNA for at least 30 PCR amplifications.
 - If NaOH is used, the isolated DNA is denatured making it highly available for PCR-amplification. The PCR reaction-mixtures should then be placed on the thermal-cycler only after the temperature has reached 72°C, or perform hot-start PCR to prevent mispriming.
 - Some PCR reactions are very sensitive to the amount of DNA template used. In such instances, titration of the DNA/Dynabeads® complex is recommended with comparisons made between eluted and non-eluted DNA.
 - PCR capacity is dependent upon parameters such as genome size, the complexity of the starting material etc. Generally, 200 ng of mammalian DNA is sufficient template for at least 10 PCR reactions. It is recommended that PCR-profiles with at least 30–35 cycles are used.
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Troubleshooting

Observation	Cause	Solution
Complex does not form or the complex is small and fragmented	Very little DNA is present or the DNA is degraded	<ul style="list-style-type: none"> • If working with blood, check the white blood cell count. • Increase the sample quality and quantity.
Complex is unusually large and difficult to handle	Large amount of DNA present	<ul style="list-style-type: none"> • See Sample Pre-treatment. • See Handling the DNA/Dynabeads® Complex. • If the problem persists, use less sample or more Dynabeads®.
Complex is difficult to homogenize	Complex has been insufficiently pipetted	<ul style="list-style-type: none"> • Continue pipetting until the complex is fully resuspended. • Leave for 30–60 seconds or longer and then resume pipetting (see Handling the DNA/Dynabeads® Complex).
	Aperture on the pipette tip is too large	Use a pipette tip with a narrower aperture.
No PCR amplification observed	Presence of PCR inhibitors	<ul style="list-style-type: none"> • Ensure that the Washing Buffer is brought to room temperature. • Add Washing Buffer more vigorously. • Ensure that the supernatant is completely removed at each washing step. • Introduce an additional (third) washing step. • Use less starting material for the PCR. • Titrate the amount of Mg²⁺, enzyme and dNTP used.
	Insufficient PCR cycles	Increase the number of PCR cycles.

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Troubleshooting, continued

Observation	Cause	Solution
Number of PCR reactions possible is lower than expected	Fragmentation of the complex during the washing steps, washing may have been too vigorous	Add Washing Buffer less vigorously.
	Inefficient elution	<ul style="list-style-type: none"> • Run out some of the Dynabeads® on an agarose gel. If DNA is found to be predominantly on the beads, then elution was inefficient. • Ensure that all Washing Buffer is removed before adding Resuspension Buffer. • Increase the force used to homogenize the complex. • Increase sample quality and quantity. • Increase the number of PCR cycles.
PCR background is too high	Concentration of template-DNA, PCR-primers, Mg ²⁺ or dNTPs may be too high	<ul style="list-style-type: none"> • Place the PCR reaction-mixtures on the thermalcycler after the temperature has reached 72°C or perform hot-start PCR. • Reduce the amount of template-DNA, PCR-primers, enzyme and Mg²⁺ used. • Elute the DNA prior to PCR amplification.
	Isolated DNA may still contain contaminants.	<ul style="list-style-type: none"> • Add Washing Buffer more vigorously. • Ensure that the supernatant is completely removed at each washing step.

Appendix

Accessory Products

Introduction The following products may be used with the Dynabeads® DNA DIRECT™ Universal Kit. For details, visit www.lifetechnologies.com or contact **Technical Support** (see page 26).

Product	Quantity	Cat. no.
Dynabeads® DNA DIRECT™ Blood Kit	100 isolations	63102
DynaMag™ -2	each	12321D
SampleRack (for DynaMag™ -2)	each	12322D
DynaMag™-96 Side Skirted	each	12027
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**.

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- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
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 - 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

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**General
Information**

Life Technologies AS complies with the Quality System Standards ISO 9001:2008 and ISO 13485:2003.

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