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Dynabeads® SILANE genomic DNA Kit

Catalog no. 37012D **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 004)

Kit Contents

Kit contents	Volume
Dynabeads® MyOne™ SILANE	5 mL
Lysis/binding buffer	35 mL
Washing Buffer 1	2 × 60 mL
Washing Buffer 2	2 × 30 mL
Elution Buffer	10 mL

Isolates genomic DNA from ~35 mL whole blood

Dynabeads® MyOne™ SILANE contains 40 mg beads/mL in purified water with 0.02% sodium azide as a preservative.

Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

See "Description of Materials" for Buffer content.

Product Description

The Dynabeads® SILANE genomic DNA kit is designed for highly predictable and consistent isolation of nucleic acids. The specific characteristics of the beads and buffers in the kit are optimized for isolation of genomic DNA from human blood. As an indication of the excellent performance of this kit, one isolation using 50 µL (2 mg) beads will typically isolate 10 µg DNA from a 350 µL blood sample ($_{A260/280}$ ≥1.8, A260/230 ≥1.4). The kit contains beads and buffers sufficient for 96 isolations.

The Dynabeads® MyOne™ SILANE suspension is the key component of this kit. The Dynabeads® MyOne™. SILANE component of this kit is available as a separate product, and is available in bulk quantities on an OEM-basis.

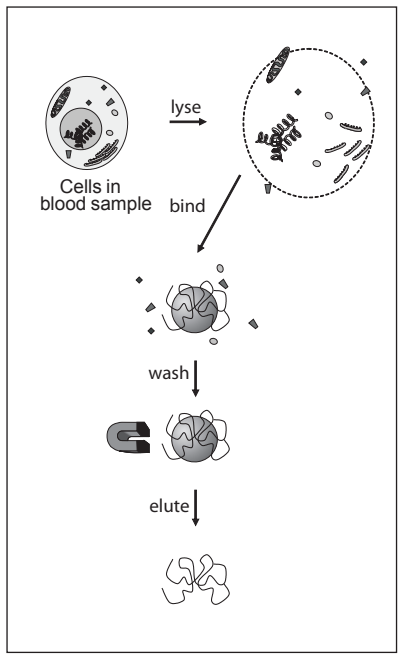

Figure 1: Illustration showing magnetic separation of genomic DNA from human blood using the Dynabeads® SILAN genomic DNA kit.

Principle

The Dynabeads® SILANE genomic DNA kit provides an excellent tool for isolation of genomic DNA from human blood, following a simple separation protocol (fig. 1).

A buffer is first added to the sample for lysis, followed by incubation with the beads. The beads will bind to the DNA in the sample and will easily and rapidly be separated by using a magnet. The magnetic separation also facilitates simple washing and elution of the isolated DNA. Dynabeads® magnetic separation technology is easily adapted to automated liquid handling platforms.

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Proteinase K at a concentration of 20 mg/mL (e.g. Sigma Cat. no. P2308, dissolved in 10 mM Tris-HCl, pH 8).
- Isopropanol (≥99.5%).
- Ethanol (96–100%).

General Guidelines

- Use a mixer that provides tilting and rotation of the tubes to ensure that Dynabeads® do not settle in the tube.
- This product should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than recommended volume of Dynabeads®.
- Carefully follow the recommended pipetting volumes and incubation times.
- Do not add Proteinase K directly into Lysis/Binding Buffer (genomic DNA).
- All vortex steps should be performed at maximum speed.
- The vial containing Dynabeads® MyOne™ SILANE should be resuspended (e.g. vortex) to a homogenous suspension prior to use. Leave the vial on a roller until use.
- For adaptation to automated format the vortex steps can be replaced by pipette action.

Protocol

The following protocol illustrates isolation of genomic DNA from 350 µL of human blood using 50 µL (2 mg) Dynabeads® MyOne™ SILANE. The protocol can be scaled up or down to suit specific needs (sample volumes, elution volumes, etc.).

Prepare the Buffers

Washing Buffer 1 (genomic DNA) and Washing Buffer 2 (genomic DNA) are supplied as concentrates. To obtain working solutions, isopropanol/ ethanol must be added to the two buffers as indicated on the respective labels.

- Prior to first time use, add 40 mL isopropanol to each of the two supplied bottles of Washing Buffer 1 (genomic DNA) to obtain a working solution.
- Prior to first time use, add 70 mL ethanol to each of the two supplied bottles of Washing Buffer 2 (genomic DNA) to obtain a working solution.
- Proceed with isolation of genomic DNA as described in the following protocol.

Isolate Genomic DNA

1. Add 50 µL Proteinase K (20 mg/mL) to a 350 µL blood sample. Vortex 30 sec (all vortex steps should be performed with maximum speed). Incubate for 2 min at room temperature.
2. Add 350 µL Lysis/Binding Buffer (genomic DNA) to the sample and vortex 15 sec to mix. Incubate for 5 min at 55˚C and then vortex for 15 sec. Incubate again for further 5 min at 55˚C followed by a final vortex for 15 sec.
3. Add 50 µL resuspended Dynabeads® MyOne™ SILANE suspension and vortex for 5 sec to mix. Add 400 µL isopropanol (100%). Vortex 30 sec and incubate on a roller for 3 min at room temperature followed by a final vortex for 30 sec.
4. Place the tube on the magnet and let the Dynabeads® collect at the magnet for 2 min. Remove the supernatant by using a pipette. The Dynabeads®/gDNA will form a nice pellet at the side of the tube, avoid touching this pellet with the pipette.
5. Remove the magnet and add 950 µL Washing Buffer 1 (genomic DNA). Vortex 30 sec at room temperature. The bead pellet should easily break into barely visible aggregates.
6. Place the tube on the magnet for 2 min. Remove the supernatant.
7. Repeat steps 5 and 6.
8. Add 950 µL Washing Buffer 2 (genomic DNA). Vortex 30 sec at room temperature to resuspend the Dynabeads®, and transfer the resuspended bead-solution to a clean tube.
9. Place the tube on the magnet for 1 min. Remove the supernatant.
10. Add 950 µL Washing Buffer 2 (genomic DNA) and vortex 30 sec.
11. Place the tube on the magnet for 1 min. Remove the supernatant. (Make sure that all the supernatant is completely removed in this last washing step. Use a small pipette tip and make sure that no droplets are left on the tube wall). Leave the tube on the magnet and let the bead pellet dry for 5 min at room temperature.
12. Remove the magnet and add 100 µL Elution Buffer (genomic DNA). Completely resuspend the Dynabeads® by vortexing and/or pipetting for 2 min at room temperature.
13. Place the tube on the magnet and let the Dynabeads® collect at the magnet for 1 min.
14. Transfer the supernatant containing the purified genomic DNA to a clean tube.

Description of Materials

Dynabeads® MyOne™ SILANE are uniform, superparamagnetic beads (1 µm in diameter) with an optimized silica-like chemistry on the bead surface. The increased magnetic strength of these beads ensure rapid magnetic mobility and efficient isolation of DNA and the low sedimentation rate and favorable reaction kinetics makes them particularly suited for automated assays. The Lysis/Binding Buffer 1 contains a guanidine salt. See the Safety Data Sheet for further safety information. All kit reagents are of analytical grade.

Related Products

Product	Cat. no.
DynaMag™-2	12321D
DynaMag™-5	12303D
HulaMixer® Sample Mixer	15920D
Dynabeads® MyOne™ SILANE	37002D
Dynabeads® MyOne™ SILANE viral NA kit	37011D

REF on labels is the symbol for catalog number.

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