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Dynabeads® SILANE viral NA

Catalog no. 37011D **Store at 2˚C to 8˚C**

Pub. no. MAN0008463 **Rev. Date:** August 2013 (Rev. 3.0)

Kit Contents

Kit contents	Volume
Dynabeads® MyOne™ SILANE	5 mL
Lysis/binding Buffer (viral NA)	30 mL
Washing Buffer 1 (viral NA)	2 × 45 mL
Washing Buffer 2 (viral NA)	30 mL
Elution Buffer (viral NA)	10 mL

Isolates DNA/RNA from ~20 mL serum/plasma (~96 reactions).

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 viral NA kit is designed for highly predictable and consistent isolation of viral nucleic acids (viral NA). The specific characteristics of the beads and buffers in the kit are optimized for sensitive isolation of viral DNA and RNA from human serum/plasma samples. The kit also allows for effective isolation of nucleic acids from challenging viruses (e.g. HBV). As an indication of the enhanced sensitivity relative to alternative magnetic separation products, the Dynabeads® SILANE viral NA kit can detect HBV DNA in a 200 µL serum sample containing only 40 infectious units. The kit contains beads and buffers sufficient for 96 isolations.

See "Description of Materials" for more information about the beads. The Dynabeads® MyOne™ SILANE component of this kit can also by purchased separately and is available in bulk quantities on an OEM-basis upon request.

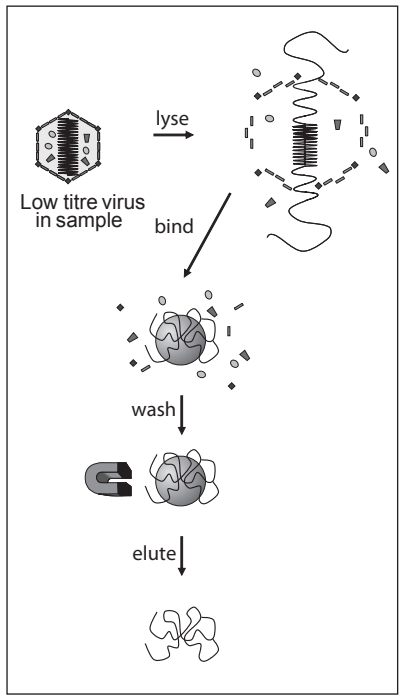

Figure 1: Illustration showing magnetic separation of viral nucleic acids using the Dynabeads® SILAN viral NA kit.

Principle

The Dynabeads® SILANE viral NA kit provides an excellent tool for sensitive isolation of viral nucleic acids, following a simple separation protocol (fig. 1).

A buffer is first added to the serum/plasma sample for lysis, followed by incubation with Dynabeads® MyOne™ SILANE. The beads with bound DNA/RNA are easily pulled to the side of the test tube by using a magnet and unbound material is removed by aspiration. The magnetic separation also facilitates simple washing and elution of the isolated DNA/RNA.

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® magnetic beads do not settle in the tube.
- Do not use this product with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Never use less than recommended volume of Dynabeads® magnetic beads.
- Carefully follow the recommended pipetting volumes and incubation times.
- Do not add Proteinase K directly into Lysis/Binding Buffer (viral genomic DNA) without serum/plasma.
- 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 viral DNA/RNA from 200 µL of serum/plasma using 50 µL (2 mg) Dynabeads® MyOne™ SILANE. The protocol can be scaled up or down to suit specific needs (sample volumes, elution volumes).

Prepare the Buffers

Washing Buffer 1 (viral NA) and Washing Buffer 2 (viral NA) 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 45 mL isopropanol to each of the two supplied bottles of Washing Buffer 1 (viral NA) to obtain a working solution.
- Prior to first time use, add 70 mL ethanol to Washing Buffer 2 (viral NA) to obtain a working solution.

Note: The Lysis/Binding Buffer (viral NA) and the concentrated stock solutions of Washing Buffer 1 (viral NA) will form a precipitate upon storage at temperatures below 15°C. Dissolve on a roller at 37°C for 1 hour, and place at room temperature prior to use. Store away from light.

Isolate viral DNA/RNA

1. Add 50 µL Proteinase K (20 mg/mL) to an empty tube, then add 200 µL serum/plasma and mix by gently tapping the tubes.
2. Add 300 µL Lysis/Binding Buffer (viral NA). Mix by inverting the tubes 4–6 times and incubate at room temperature for 5 minutes.
   Note: Do not add Proteinase K directly into Lysis/Binding Buffer (viral NA) without serum/plasma.
3. Add 150 µL isopropanol. Then add 50 µL resuspended Dynabeads® MyOne™ SILANE suspension to the mixture from step 2. Mix and incubate on a roller at room temperature for 10 minutes.
4. Place the tube on the magnet and let the Dynabeads® magnetic beads collect at the magnet for 2 minutes (or until the supernatant is clear). Remove the supernatant by using a pipette.
5. Remove the magnet and add 850 µL Washing Buffer 1 (viral NA). Resuspend the Dynabeads® magnetic beads by inverting the tubes or pipetting up and down 3–4 times.
6. Place the tube on the magnet for 1 minute. Remove the supernatant.
7. Repeat steps 5 and 6.
8. Remove the magnet and add 450 µL Washing Buffer 2 (resuspend the Dynabeads® magnetic beads by pipetting). Transfer the resuspended bead-solution to a clean tube.
9. Place the tube on the magnet for 2 minutes. Remove the supernatant.
10. Repeat steps 8 and 9. (A second change of tube is not required.) Make sure that all buffer is completely removed in this last washing step. (Tip: While still on the magnet, tap the tube/magnet on the table to collect the last drop at the bottom of the tube. 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 beadpellet dry at room temperature for 10–15 minutes.
11. Remove the magnet and add 100 µL Elution Buffer (viral NA). Resuspend the Dynabeads® magnetic beads by pipetting and incubate at 70°C for 3 minutes.
12. Resuspend the Dynabeads® magnetic beads by gently tapping the tubes. Place the tube on the magnet and let the Dynabeads® collect at the magnet for 2 minutes.
13. Transfer the supernatant containing the purified viral DNA/RNA 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 and Washing Buffer 1 contains a guanidine salt. See the Safety Data Sheet for further safety information. The Washing Buffer 2 and Elution Buffer provided in the kit are produced and packed under RNase-free conditions. All kit reagents are of analytical grade.

Related Products

Product	Cat. no.
DynaMag™-2 Magnet	12321D
DynaMag™-5 Magnet	12303D
HulaMixer® Sample Mixer	15920D
Dynabeads® MyOne™ SILANE	37002D
Dynabeads® MyOne™ SILANE genomic DNA	37012D

REF on labels is the symbol for catalog number.

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