OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Tissue DNA KF 96 Kit

M6329-00	1 x 96 preps
M6329-01	4 x 96 preps
M6329-02	20 x 96 preps

July 2012

For research use only.Not intended for diagnostic testing.

Mag-Bind® Tissue DNA KF 96 Kit

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Manual Revision: July 2012

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Introduction

The Mag-Bind® Tissue DNA KF 96 kit is designed for rapid and reliable isolation of high-quality genomic DNA from a wide variety of tissues and cultured cells. Up to 10 mg animal tissue or 5 x 10^6 cells can be processed in less than 1 hour. The Mag-Bind® magnetic beads technology provides high-quality DNA, which is suitable for direct use in most downstream applications such as amplifications and enzymatic reactions. This kit can be easily adapted with automated system and the procedure can be scaled up or down, allowing purification from various amounts of starting materials.

Overview

If using the Mag-Bind® Tissue DNA 96 KF Kit for the first time, please read this booklet to become familiar with the procedure and its various modification. Animal tissue or culture cells are pre-treated with proteinase K and then lysed in a specially formulated buffer containing detergent. DNA was bound to the surface of Mag-Bind® magnetic particles. Proteins, polysaccharides, and cellular debris are efficiently washed a with few wash steps. Pure DNA is then eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

New in this Edition: This manual has been edited for content and redesigned to enhance user readability.

- Proteinase K is now supplied in a liquid form eliminating the step to resuspend prior to use.
- Proteinase K Solution can be stored at room temperature for 12 months.
- Proteinase Storage Buffer is no longer included in the kit.

Kit Contents

Product	M6329-00	M6329-01	M6329-02
Preparations	1 x 96	4 x 96	20 x 96
Mag-Bind® Particles CND	1.1 mL	4.4 mL	22 mL
MSL Buffer	35 mL	140 mL	700 mL
TL Buffer	35 mL	120 mL	600 mL
Binding Enhancer	1.1 mL	4.4 mL	22 mL
SPM Buffer	36 mL	144 mL	3 x 300 mL
QMP Buffer	30 mL	120 mL	2 x 300 mL
Elution Buffer	15 mL	60 mL	2 x 150 mL
Proteinase K Solution	2.2 mL	9 mL	44 mL
User Manual	✓	✓	✓

Storage and Stability

Most components of the Mag-Bind® Tissue DNA KF 96 Kit can be stored at room temperature. Proteinase K Solution can be stored at room temperature for 12 months. For long-term storage (>12 months), store Proteinase K Solution at 2-8°C. Binding Enhancer and Mag-Bind® Particles CND must be stored at 2-8°C. Under these conditions, performance of all components of the kit are guaranteed at least 12 months. Under cool ambient conditions, a precipitate may form in the MSL and TL Buffers. In case of such an event, heat the bottle at 37°C to dissolve the precipitate.

Preparing Reagents

1. Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M6329-00	84 mL
M6329-01	336 mL
M6329-02	700 mL per bottle

2. Dilute QMP Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
M6329-00	30 mL
M6329-01	120 mL
M6329-02	300 mL per bottle

KingFisher Pipetting Instructions

Pipetting Instructions for KingFisher 96 and Mag-Bind® Tissue DNA KF 96 protocols

Plate Type*	Plate	Content	Reagent Volume
A	1	sample/lysate	
A	2	QMP Buffer	500 µL
A	3	SPM Wash Buffer	600 µL
A	4	SPM Wash Buffer	600 µL
B	5	Elution Buffer	100 µL**
A	6	Tip Sleeve	

**** A= KingFisher 96-well deep-well plate, B=KingFisher 96 KF plate***
*****When using samples of Spleen, Kidney, Liver or Lung tissues, Elution Buffer should be adjusted to 200-400 µL for optimal results.***

- Prepare sample/lysate by following the instructions based on sample type.
- Add 500 µL QMP Buffer to Plate 2.
- Add 600 µL SPM Wash Buffer to Plate 3.
- Add 600 µL SPM Wash Buffer to Plate 4.
- Add 100 µL Elution Buffer to Plate 5.

Recommended Program

The Following recommendation are based upon Thermo Fisher's KingFisher Software Version 2.6.2. Contact the instrument's manufacturer for updated software if all speeds are not available. This protocol is designed to show the recommended settings for all binding, washing, drying, and elution steps in a 96-well format. Lysis steps are not included in this protocol.

Step 1: Binding
Beginning of Step: No Action
Binding Parameters: 05:00 at Superfast;
Collect Beads at Count of 8

Step 2: High Salt Wash Step
Beginning of Step: Release Beads, 00:10 at Fast,
Wash Time: 01:00, Very Fast;
Collect Beads at Count of 8

Step 3: Ethanol Wash Step
Beginning of Step: Release Beads, 00:10 at Fast,
Wash Time: 1:00, Half Mix
Collect Beads at Count of 8

Step 4: Ethanol Wash Step
Beginning of Step: Release Beads, 00:10 at Fast,
Wash Time: 1:00, Half Mix
Collect Beads at Count of 8

Step 5: Dry Step
05:00 Outside Wells/tubes

Step 6: Elution
Beginning of Step: Release Beads 00:10 at Fast
Elution Time: 00:00 at Fast
Heating Parameters: Preheat, 08:00 at 70°C
Heating Action: Mix at Fast

Post Mix: 02:00 at Grind Mix,
Collect Beads at Count of 8
Disposal Plate: Plate Ethanol Wash 1

Mag-Bind® Tissue DNA KF 96 Kit - Whole Tissue Protocol

Materials and Reagents to be Supplied by User:

- Centrifuge capable of at least 3,000 x *g*
- Rotor adapter for 96-well microplates
- Shaking water bath or incubator capable of 56°C
- 100% Ethanol
- 8- or 12-channel pipette
- Reagent reservoirs
- KingFisher 96 or KingFisher Flex 96 with deep-well magnet
- KingFisher 96 deep-well plates
- KingFisher 96 plates
- Tip sleeve for KingFisher 96 deep-well magnet
- Sealing film
- Optional: E-Z 96® Lysate Clearance Plate (Cat# FL9601)

Before Starting:

- Set Incubator to 56°C
- Prepare Reagents according to Page 4

1. Mince up to 10 mg of tissue or two pieces of mouse tail (0.2 - 0.5 cm in length) into a 96-well deep-well plate.

2. Add 250 µL TL Buffer.

   Note: Cut the tissue into small pieces to speed up lysis.

3. Add 20 µL Proteinase K Solution. Seal the plate with sealing film. Vortex to mix thoroughly.

4. Incubate at 56°C for 1-3 hours in a shaking water bath.

   Note: If a shaking water bath is not available, incubate the plate in an incubator and vortex the plate every 20-30 minutes. Lysis time depends on the amount and type of tissue but is usually less than 3 hours. Lysis can proceed overnight.

Important: Some samples may contain tissues types, such as hair or cartilage, that can not be completely digested with Proteinase K. Those tissues will interfere with the DNA binding and bead collection causing lower DNA yield and quality. To ensure that the tissue lysate is completely free of those tissues, it is strongly recommended to use Omega Bio-tek's E-Z 96® Lysate Clearance Plate (Cat# FL9601) as follows below.

1. Place a Lysate Clearance Plate on a new KingFisher 96 deep-well plate.
2. Transfer the tissue lysate from Step 4 to the KingFisher 96 deep-well plate.
3. Centrifuge at 2,500 x *g* at room temperature for 2 minutes.
4. Proceed to Step 5.

5. Centrifuge at 3,000 x *g* for 5 minutes at room temperature.

6. Transfer 200 µL cleared lysate into a new KingFisher 96 deep-well plate. Continue to step 4.

   Note: Due to the variation of the water contents of sample, the volume of tissue lysate will vary. Always check the volume of the cleared lysate from KingFisher 96 deep-well plate to ensure the lysate volume is around 200 µL. If the volume of cleared lysate is significantly greater or less than 200 µL, adjust the volume of MSL Buffer and ethanol proportionally in the downstream steps. For example: for 220 µL tissue lysate, use 220 µL MSL Buffer and 300 µL ethanol.

Optional: Add 5 µL RNase A (25 mg/mL, assuming a sample size of 20 mg) and incubate at room temperature for 2 minutes. Proceed to Step 7.

7. Add 200 µL MSL Buffer. Vortex to mix thoroughly.

   Note: If the expected DNA yield is <1 µg, add 10 µL Binding Enhancer.

8. Add 275 µL 100% ethanol and 10 µL Mag-Bind® Particles CND.

   Note: Vortex Mag-Bind® Particles CND for 3 minutes before adding to the sample or mastermix (below).

9. Press start on KingFisher 96 Tissue Protocol and load plates accordingly. See Pipetting Instructions on Page 5.

Mag-Bind® Tissue DNA KF 96 Kit - Cultured Cell Protocol

This protocol is designed for isolation of up to 25 µg genomic DNA from 5 x 10^6 cultured cells. To increase throughput, the incubation step at 55°C for 10 minutes performed on the KingFisher can be performed externally. Contact Omega Bio-tek for a modified protocol for the KingFisher instrument.

Materials and Reagents to be Supplied by User:

- 100% Ethanol
- PBS (4°C)
- 8- or 12-channel pipette
- Reagent reservoirs
- KingFisher 96 or KingFisher Flex 96 with deep-well magnet
- Deep-well KingFisher 96 plates
- KingFisher 96 plates
- Tip sleeve for KingFisher 96 deep-well magnet
- Sealing film

Before Starting:

- Prepare Reagents according to Page 4

1. Prepare the cell suspension using one of the following depending on the starting material:

    - Frozen cell samples:

        1. Thaw cells.
        2. Pellet the cells.
        3. Wash the cells with cold (4°C) PBS.
        4. Resuspend cells in 180 µL cold PBS.
        5. Proceed to Step 2.

    - For cells grown in suspension:

        1. Harvest 5 x 10^6 cells.
        2. Centrifuge at 1,200 x *g*.
        3. Discard the supernatant.
        4. Wash the cells with cold (4°C) PBS.
        5. Resuspend cells in 180 µL cold PBS.
        6. Proceed to Step 2.

- For cells grown in a monolayer:
  1. Harvest the cells by either using a trypsin treatment or scraping with rubber policemen.
  2. Wash the cells with cold (4°C) PBS.
  3. Resuspend cells in 180 µL cold PBS.
  4. Proceed to Step 2.

2. Transfer the sample to a KingFisher 96 deep-well plate.

3. Add 20 µL Proteinase K Solution. Seal the plate with sealing film. Vortex to mix thoroughly.

4. Add 200 µL MSL Buffer.

   Note: If the expected DNA yield is <1 µg, add 10 µL Binding Enhancer.

Optional: Add 5 µL RNase A (25 mg/mL) and incubate at room temperature for 2 minutes. Proceed to Step 8.

5. Press start on KingFisher 96 Tissue Protocol and load plates accordingly. See **Pipetting Instructions (Page 6) .**

6. During the Pause Step, add 275 µL 100% ethanol and 10 µL Mag-Bind® Particles CND.

   Note: Vortex Mag-Bind® Particles CND for 3 minutes before adding to the sample. A mastermix of ethanol and Mag-Bind® Particles CND can be prepared prior to this step.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896**.

Problem	Cause	Solution
Low DNA yields	Incomplete re-suspension of magnetic particle	Resuspend the magnetic particles by vortexing before use.
	Inefficient cell lysis due to inefficient mix of MSL Buffer and sample	Make sure the sample is thoroughly mixed with MSL Buffer.
	SPM Wash Buffer was not prepared correctly.	Prepare the SPM Wash Buffer by adding ethanol according to instruction.
	Loss of magnetic beads during operation	Be careful not to remove the magnetic beads during the operation.
	Inefficient cell lysis due to decreased activity of Proteinase K	Add more Proteinase K.
Problem	Cause	Solution
No DNA eluted	SPM Wash Buffer was not diluted with ethanol	Prepare SPM Wash Buffer as instructed on Page 4.
Problem with downstream applications	Insufficient DNA was used	• Use more starting material. • Quantify the purified DNA accurately and use sufficient DNA.
	Excess DNA was used for downstream application	Make sure to use correct amount DNA.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
E-Z 96® Lysate Clearance Plate, 10 pk	FL9601
E-Z 96® Lysate Clearance Plate, 100 pk	FL9601
Multi-Channel Disposable Reservoir, 100/pk	AC1331-01
SealPlate Film, 100/box	AC1200-01
Proteinase K, 30 mg	AC110
Proteinase K, 100 mg	AC111
Proteinase K, 1 g	AC112
SPM Wash Buffer, 40 mL	PS014
Elution Buffer, 100 mL	PDR048

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