

TECHNICAL BULLETIN

MagneSil[®] Genomic, Fixed Tissue System

Instructions for Use of Product
MD1490



MagneSil[®] Genomic, Fixed Tissue System

All technical literature is available at: www.promega.com/protocols/
 Visit the web site to verify that you are using the most current version of this Technical Bulletin.
 E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

Pathologists have long used formalin-fixed, paraffin-embedded tissue samples to examine morphology. With the advent of PCR amplification, these samples provide the potential for correlating morphology with genotype. Unfortunately, the process of tissue fixation results in cross-linking between proteins and DNA and between different strands of DNA. This cross-linking reduces the efficiency of amplifying DNA and as a consequence poor results generally have been observed with this sample type.

The MagneSil[®] Genomic, Fixed Tissue System^(a) is designed for purification of DNA from many formalin-fixed samples following an overnight Proteinase K digestion. Amplified fragments up to 1.8kb in size have been successfully generated from DNA purified with this system (Figure 1). One advantage this system has over other purification methods, such as phenol-chloroform extraction, is its ability to remove most inhibitors of amplification, including very small fragments of DNA.

The MagneSil[®] Genomic, Fixed Tissue System has been developed for use with small amounts of tissue such as those found in thin sections. To help eliminate inhibitors, a small amount of DNA-binding paramagnetic resin is used, and as a result the DNA binding capacity of the system is limited to a few hundred nanograms. Use of a large amount of tissue will result in low recovery as well as poor-quality DNA when compared to other purification methods. Tissue that has been stored in formalin for extended periods of time may be too cross-linked and/or too degraded to perform as template for amplification.

1. Description (continued)

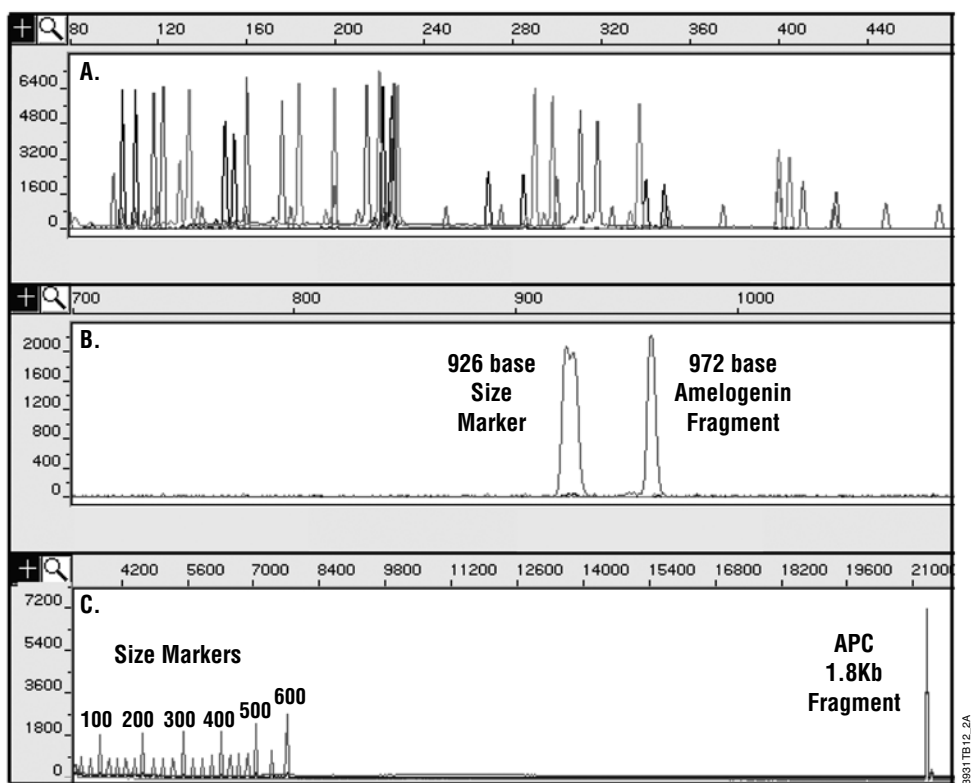


Figure 1. Analysis of DNA purified from formalin-fixed, paraffin-embedded 10µm thin sections using the MagneSil® Genomic, Fixed Tissue System. Purified DNA was amplified and the amplification products were analyzed on an ABI PRISM® 310 or 3100 Genetic Analyzer. **Panel A.** Amplification with a set of 16 fluorescently labeled primers. Amplification products range in size from 104 to 420 bases. **Panel B.** A 972-base fragment amplified using an amelogenin primer set. **Panel C.** A 1.8kb fragment amplified from the APC gene. Increasing the extension time during amplification may help to balance yields between small and large amplification products and increase yields for large amplification products. Results will vary depending on the degree of cross-linking due to formalin fixation.

For citations using the MagneSil® Genomic, Fixed Tissue System, visit: www.promega.com/citations/

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
MagneSil® Genomic, Fixed Tissue System	100 samples	MD1490

Each system contains sufficient reagents to purify 100 samples. The MagneSil® Genomic, Fixed Tissue System includes one each of the following modules:

MD1170: MagneSil® Genomic, Fixed Tissue Processing Module. Includes:

- 35ml Incubation Buffer
- 2 × 10mg Proteinase K
- 1.125ml DTT, 1M

MD1180: MagneSil® Genomic, Fixed Tissue Purification Module. Includes:

- 0.9ml Resin
- 40ml Lysis Buffer
- 30ml 2X Wash Buffer
- 15ml Elution Buffer

Note: MD1170 and MD1180 are shipped separately.

Storage Conditions: Store Proteinase K and DTT at –20°C. Store Incubation Buffer between –20°C and 25°C. Store all other reagents at room temperature. See expiration dates on module labels.

3. DNA Isolation From Formalin-Fixed, Paraffin-Embedded Tissue

Materials to Be Supplied by the User

- 95–100% ethanol
- isopropyl alcohol
- 56°C heat block, water bath or thermal cycler
- 65°C heat block, water bath or thermal cycler
- vortex mixer
- Microtubes, 1.5ml (Cat.# V1231)
- aerosol-resistant micropipette tips
- MagneSphere® Technology Magnetic Separation Stand (twelve-position) (Cat.# Z5342)



3.A. Preparation of Proteinase K, DTT, Incubation Buffer/Proteinase K Solution and 1X Wash Buffer

Stock Proteinase K Solution

1. Add 550 μ l of Incubation Buffer without DTT to each bottle of lyophilized Proteinase K and gently swirl to dissolve. The final concentration of Proteinase K will be 18mg/ml.
2. Dispense the Proteinase K solution into small aliquots. The Proteinase K solution can be frozen and thawed up to five times with no significant loss in activity. Use ten microliters per sample.

1M DTT

1. Dispense the DTT into small aliquots and freeze at -20°C .

Incubation Buffer/Proteinase K Solution

Note: This solution should be prepared fresh for each set of DNA purifications.

1. Prepare the Incubation Buffer/Proteinase K solution by combining the Incubation Buffer, 1M DTT and the stock Proteinase K solution in the indicated proportions. Prepare 100 μ l of Incubation Buffer/Proteinase K solution for each sample.

Incubation buffer	800 μ l
1M DTT	100 μ l
18mg/ml Proteinase K	100 μ l
Final volume	1ml

2. Mix gently and store on ice until ready to use.

1X Wash Buffer

1. Add 15ml of 95–100% ethanol and 15ml of isopropyl alcohol to the 2X Wash Buffer.
2. Replace cap and mix by inverting several times.
3. Mark label to record the addition of alcohols. Label bottle as 1X Wash Buffer. Store solution at room temperature. Close bottle tightly to prevent evaporation.

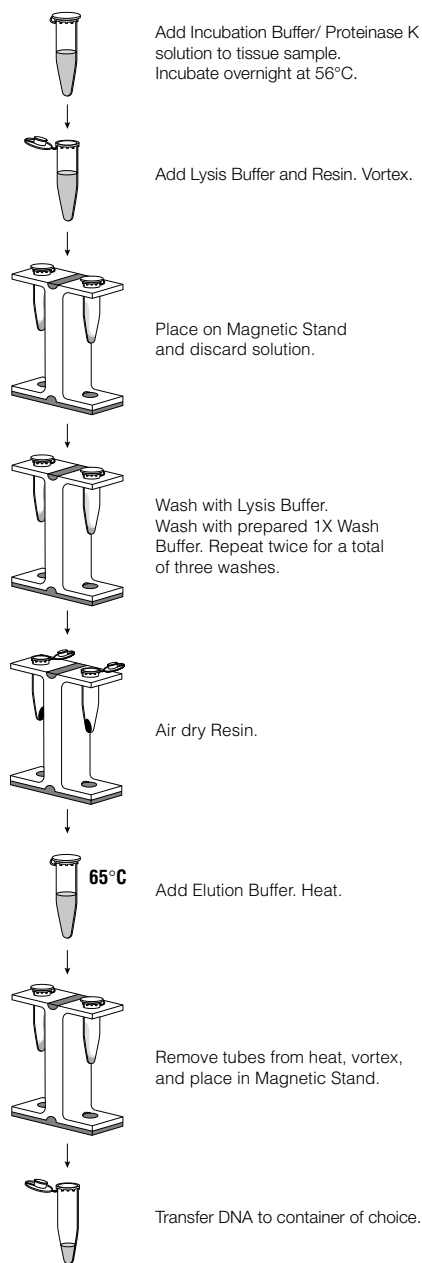


Figure 2. Schematic of DNA isolation from formalin-fixed, paraffin-embedded tissue.
See Section 3.B for a detailed protocol.

3.B. DNA Isolation from Formalin-Fixed, Paraffin-Embedded Tissue



Use of gloves and aerosol-resistant micropipette tips is highly recommended to prevent cross-contamination.

1. Place sample in a 1.5ml microcentrifuge tube (e.g., Microtubes, 1.5ml, Cat.# V1231).
2. Add 100µl of freshly made Incubation Buffer/Proteinase K solution. Incubate at 56°C overnight. There is no need to deparaffinize thin tissue sections.
3. Remove the tube and add 2 volumes of Lysis Buffer.
Note: If Lysis Buffer forms a precipitate, warm solution to 37–60°C.
4. Vortex the Resin bottle for 10 seconds at high speed or until Resin is thoroughly mixed. Add 7µl of Resin slurry to the Proteinase K-treated sample. For uniform results, keep the Resin resuspended while it is dispensed.
5. Vortex sample/Lysis Buffer/Resin mix for 3 seconds at high speed. Incubate at room temperature for 5 minutes.
6. Vortex for 2 seconds at high speed and place tube in the Magnetic Stand. Separation will occur instantly.
Note: If Resin does not form a distinct pellet on the side of the tube, vortex the tube and quickly place back in the stand.
7. Carefully remove and discard all of the solution without disturbing the Resin on the side of the tube.
8. Add 100µl of Lysis Buffer to the Resin-containing tube. Remove the tube from the Magnetic Stand and vortex for 2 seconds at high speed.
9. Return tube to the Magnetic Stand and discard all Lysis Buffer.
10. Add 100µl of prepared 1X Wash Buffer. Remove tube from the Magnetic Stand and vortex for 2 seconds at high speed.
11. Return tube to the Magnetic Stand, and carefully remove and discard all Wash Buffer.
12. Repeat Steps 10 and 11 twice more for a total of 3 washes with the prepared 1X Wash Buffer. Make sure that all of the solution has been removed after the last wash.
13. With lid open, air-dry the Resin in the Magnetic Stand for 5 minutes.



Do not dry for more than 20 minutes, as this may inhibit removal of DNA.

14. Add 25µl of Elution Buffer.
15. Close the lid, vortex the tube for 2 seconds at high speed and place it at 65°C for 5 minutes.
16. Remove the tube from the heat block and vortex for 2 seconds at high speed. Immediately place on the Magnetic Stand.
17. Carefully transfer the DNA-containing solution to a container of choice. We recommend polypropylene containers to reduce the amount of DNA that binds to the sides.

Note: The DNA solution can be stored at 4°C for short-term storage or at –20°C or –70°C for long-term storage.

4. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. E-mail: techserv@promega.com

Symptoms	Causes and Comments
Poor amplification	Sample too highly cross-linked or degraded. <ul style="list-style-type: none"> • Purify more material. • Increase extension time for each amplification cycle.
Poor yield	<p>Too much sample. The resin has capacity of a few hundred nanograms. Use less sample or more resin.</p> <p>Ethanol and isopropyl alcohol were not added to 2X Wash Buffer. Make certain that both alcohols were added to the 2X Wash Buffer. Record addition on bottle label.</p> <p>Resin was not thoroughly resuspended prior to pipetting. Vortex Resin bottle for 10 seconds at high speed or until Resin is thoroughly mixed. Keep Resin resuspended while dispensing.</p> <p>Incomplete Proteinase K digestion. Mince large tissue pieces before addition of Incubation Buffer/Proteinase K solution. The Incubation Buffer/Proteinase K solution must be made fresh for each set of DNA purifications.</p> <p>Resin was overdried after washing steps. Do not air-dry Resin for more than 20 minutes.</p>
Amplification product not visible on gel	Not enough DNA available. Use fluorescently labeled primer.
Poor Resin “pellet” formed	Resin settled before tube was placed in Magnetic Stand immediately after vortexing/mixing. Repeat vortexing/mixing and place tube back in stand.
Resin present in final eluted solution (carryover of Resin may affect results in downstream assays)	Resin can be transferred by rapid pipetting. During the elution step, Resin can also become trapped in the meniscus of the final eluant. Vortex/mix eluted solution, place in Magnetic Stand and carefully transfer eluant to new tube.



5. Related Products

Product	Size	Cat.#
MagneSphere® Technology Magnetic Separation Stand (two-position)	1.5ml	Z5332
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	1.5ml	Z5342
Microtubes, 1.5ml	1,000/bag	V1231

For a complete listing of our amplification products, visit: www.promega.com

6. Summary of Changes

The following changes were made to the 5/17 revision of this document:

1. Discontinued products were removed from Related Products.
2. The document design was updated.

®U.S. Pat. Nos. 6,027,945 and 6,368,800 and other patents pending.

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