

DNAgard™ Tissues & Cells Handbook

Preserve DNA in biological samples at room temperature

For room temperature storage and shipment of
DNA in biological samples.

Biomātrica®
THE BIOSTABILITY COMPANY

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DNAgard is designed for room temperature storage and shipment of DNA in biological samples, such as mammalian cells and tissues. DNA in complex samples is protected by the unique stabilization properties of DNAgard. Samples are kept at room temperature, removing the need for frozen storage or immediate processing. DNAgard is easy to use - samples are applied into the DNAgard solution, mixed, and stored. Samples can be stored in liquid DNAgard for 2 months at room temperature. DNAgard also offers the unique option to store samples in a dried format for long-term stability. Samples stored in liquid DNAgard are ready for immediate processing for DNA recovery via column extraction (following manufacturer's instructions) or using standard lab procedures involving digestion and organic extraction. Dried samples need only be rehydrated in water or buffer and processed by DNA isolation columns. Once purified, DNA can be used directly in downstream applications.

Kit Components

- DNAgard (50 ml or 100 ml bottle)
- Protocol

Storage

Prior to use, DNAgard must be stored at room temperature. Use within 6 months of purchase date for optimal product performance.

DNAgard stabilizes genomic DNA in cultured cells and animal tissue samples for 2 months at room temperature in a liquid storage format. Longer-term stabilization is achieved by drying samples in DNAgard. Dried samples are stored at room temperature in a sealed moisture-barrier bag containing 9 g of desiccant. *Foil bags and desiccant packets are available for purchase, visit www.biomatrix.com.*

Product Use Limitations

The DNAgard test kit is for performance evaluation only. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of disease.

Safety Information

DNAgard contains a chaotropic salt that is harmful if swallowed and irritating to eyes and skin. Keep away from food and drink. Wear suitable protective clothing and gloves. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. If swallowed, seek medical advice immediately.

Protocols for DNAgard - Liquid Format

SAMPLE STORAGE

Storage of Tissue Samples in DNAgard – Liquid storage

1. **Prepare tissue samples by dissection.** For optimal DNA protection, store tissue fragments less than 75 mg. Small tissue fragments, thinly sliced (at least one edge of the tissue fragment be 5mm or less in length), ensures that DNAgard permeates rapidly the entire tissue sample. To maintain the integrity of the DNA, tissue fragments should be kept cold during dissection and transferred to DNAgard as soon as possible.
2. **Submerge tissue fragment in 500 µl (at least 100 µl per 10 mg of tissue are required) DNAgard solution for shipment or storage.** If sample is to be shipped, it is important to select a tube size that ensures that the tissue remains submerged during handling. We recommend the use of screw-cap tubes to prevent sample leakage during transport. For general storage, use 2 ml screw-cap tubes.
3. **Store samples at room temperature and protected from light for 2 months.**

(OPTIONAL) Transfer of samples from liquid storage to dry storage

Optionally, if samples were previously stored in liquid DNAgard for 30 days or less, these samples can be dried down for longer storage period. To do this, simply follow the relevant protocol for dry storage (see p10).

Storage of Tissue Culture Cells in DNAgard– Liquid storage

Genomic DNA from tissue culture cells is released into the DNAgard solution during storage and is stabilized for two months. DNA extraction methods using phenol-chloroform are not compatible with DNAgard solution. Column-based DNA isolation methods must be used for cells stored in DNAgard.

1. Aliquot cells for shipment or storage.

DNAgard has been shown to protect genomic DNA in sample concentrations ranging from 2×10^3 to 10^6 cells per 100 μ l DNAgard.

We recommend the use of screw-cap tubes to prevent sample leakage during transport.

When choosing the number of cells to store, consider the desired DNA yield and the specifications of the DNA isolation procedure used to recover the genomic DNA. Refer to the manufacturer's specifications in the DNA isolation protocol for product limitations and expected yields (in general, approximately 200 – 600 ng of nucleic acid can be recovered from 10^5 cultured cells containing a normal set of chromosomes).

2. Pellet cells at 3500xg for 1-2 minutes in a microcentrifuge (volumes exceeding 1.5 ml may require longer spin times). Remove supernatant.

3. Add a minimum of 100 μ l of DNAgard solution for every 10^6 cells (preferably, use screw cap tubes to avoid spillage).

Pulse-vortex samples to resuspend pellet. If the cell suspension in DNAgard is too viscous to pipet, then add more DNAgard solution. Avoid adding excessive amounts of DNAgard, as this will increase the volumes of reagents required for DNA recovery.

4. Store samples at room temperature and protected from light for 2 months.

(OPTIONAL) Transfer of samples from liquid storage to dry storage

Optionally, if samples were previously stored in liquid DNAgard for 30 days or less, these samples can be dried down for longer storage period. To do this, simply follow the relevant protocol for dry storage (see **p13**).

SAMPLE RECOVERY

General Note: This product is designed for optimal DNA recovery using commercially available DNA isolation column technologies or (for tissue samples) standard, organic extraction methods.

Sample recovery from tissue samples stored in DNAgard – liquid storage

The genomic DNA of samples stored in liquid DNAgard is stable for 2 months at room temperature.

1. Process samples for DNA recovery via commercially available column purification technologies (1a, 1b, 1c, Appendix A) or via standard laboratory procedures involving tissue lysis and organic extraction (1d). **For ease of use, we recommend removing the DNAgard solution from the tissue fragment prior to DNA isolation. However, if DNA yield is critical for you,** optimal DNA recovery is achieved by isolating genomic DNA from the entire DNAgard sample (tissue fragment plus DNAgard solution – see sections 1a and 1 b below and Appendix A).

Note on tissue disruption: DNA isolation via commercially available column technologies can often be facilitated by disruption of the tissue sample, thereby reducing the time required to fully lyse the tissue and release genomic DNA. We recommend the use of a pestle. Consult the DNA isolation manufacturer's instructions.

- a. **Removal of DNAgard solution for DNA purification using column technologies.**

In cases where maximal DNA yield is not critical or a reduction in reagent volumes is desired, DNAgard solution can be pipetted off prior to DNA extraction using column purification kits. **Take care to avoid pipetting off tissue fragments.** Process the tissue fragment for DNA isolation according to the kit manufacturer's instructions.

- b. **Maximal DNA recovery of genomic DNA.** For column purification protocols allowing DNA isolation from tissue samples resuspended in buffer, no additional processing of DNAgard samples is needed. Follow manufacturer's instructions for DNA isolation, adhering to buffer-to-sample ratio specifications.

Note: Check with the manufacturer's instructions for DNA isolation to determine reagent volumes needed to process the DNAgard sample. It is often necessary to transfer the sample, after tissue

lysis, to a larger capacity tube (*i.e.* > 2 ml) or to divide the sample into multiple tubes.

We have developed an alternate protocol for the QIAamp DNA Mini Kit (Qiagen) to minimize reagent volumes needed for DNA isolation from tissues stored in liquid DNAgard. See Appendix A.

- c. Maximal DNA recovery of genomic DNA – modified protocol** for column purification kits **that initiate from** a tissue sample free of liquid. Simply add the kit's initial lysis buffer in a 1:1 ratio with the DNAgard volume. Scale all other reagents as necessary based on this initial volume (proceed as if the resultant mixture was entirely kit lysis buffer) and process according to the kit specifications. (We have verified this modified protocol with the following DNA isolation kits: GE illustra tissue and cells genomicPrep Mini Spin Kit, Invitrogen PureLink Genomic DNA mini Kit, MO BIO UltraClean Tissue & Cells DNA Isolation Kit and QIAGEN QIAamp DNA Mini Kit).

An example of a modified protocol for DNA extraction from tissue **stored in liquid DNAgard using the QIAGEN QIAamp DNA Mini Kit is provided in Appendix A.** This protocol minimizes the reagent volumes required for DNA isolation.

- d. DNA isolation involving organic extraction.** Tissues stored in DNAgard can be processed for DNA isolation using standard lab protocols involving tissue lysis and organic extraction. Simply pipette off the DNAgard solution, being careful to not remove the tissue sample. Add lysis buffers and enzymes according to protocol.

Sample recovery from tissue culture cells stored in DNAGard – liquid storage

The genomic DNA of samples stored in liquid DNAGard is stable for 2 months at room temperature. Samples can be processed for DNA recovery directly via commercially available column purification technologies – no additional processing of DNAGard samples is needed. DNAGard solution should not be removed prior to sample processing; add lysis buffers and enzymes provided in your DNA isolation kit directly to the DNAGard sample. Follow manufacturer's instructions for DNA isolation, adhering to buffer-to-sample ratio specifications. Do not use organic extraction methods for DNA isolation (*i.e.* phenol-chloroform extraction).

If using a DNA isolation kit that specifies a protocol for cells resuspended in buffer or media, we recommend following the instructions in section 1 below. (*e.g.* QIAGEN QIAamp DNA Mini Kit; Invitrogen PureLink Genomic DNA Mini Kit).

If using a DNA isolation kit that does not specify a protocol for cells resuspended in buffer (if there is only a protocol initiating from a cell pellet) follow the instructions in section 2 below. (*e.g.* GE Illustra, MO BIO UltraClean).

1. Column purification protocols allowing DNA isolation from cells resuspended in buffer or media:

In this case, DNAGard can be treated as if it were any resuspension buffer or media. Follow manufacturer's instructions for DNA isolation, adhering to reagent ratio specifications.

Note: Check with the manufacturer's instructions for DNA isolation to determine reagent volumes needed to process the DNAGard sample. Depending on the initial volume of DNAGard, it is sometimes necessary to transfer the sample to a larger capacity tube (*i.e.* > 2 ml) or to divide the sample into multiple tubes.

2. Using column purification protocols that do not specify DNA isolation from cells resuspended in buffer or media:

Do not pellet the DNAGard-cell suspension (genomic DNA from cells is released into the DNAGard solution during storage).

Simply add the kit's initial lysis buffer in a 1:1 ratio with the DNAGard volume. Scale all other reagents as necessary based on this initial volume (proceed as if the resultant mixture was entirely kit lysis buffer) and process according to the kit specifications. (We have verified this modified protocol with the following DNA isolation kits: GE Illustra Tissue and Cells Genomic Prep Mini Spin Kit and MO BIO UltraClean Tissue and Cells DNA Isolation Kit).

An example of a modified protocol for DNA extraction from mammalian tissue culture cells **stored in liquid DNAgard using the GE illustra tissue and cells genomicPrep Mini Spin Kit is provided in Appendix C.**

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Note: 2 ml tubes should be of sufficient volume in most DNA isolation protocols. However, check with the manufacturer's instructions to insure that this is the case. If not, divide the DNAgard sample into multiple tubes prior to processing.
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Protocols for DNAgard – Dry Format

SAMPLE STORAGE

Storage of Tissue Samples in DNAgard – Dry storage

Note: Use of the dry-down option for animal tissues stored in DNAgard requires the use of a commercially available column purification kit. This storage method is not compatible with DNA isolation protocols using organic extraction (i.e. phenol-chloroform).

- 1. Prepare tissue samples by dissection.** For optimal DNA protection, store tissue fragments less than 75 mg. To maintain the integrity of the DNA, tissue fragments should be kept cold during dissection and transferred to DNAgard as soon as possible.

When choosing the amount of tissue to store, consider the desired DNA yield and the specifications of the DNA isolation procedure used to recover the genomic DNA. Refer to the manufacturer's specifications in the DNA isolation protocol for product limitations and expected yields (in general, approximately 0.1 – 1.2 µg of nucleic acid can be recovered from 1 mg tissue).

- 2. Submerge tissue fragment in 500 µl DNAgard solution (at least 100 µl per 10 mg of tissue are required).** Any tube can be used, however be sure to consider compatibility with the tissue disruption step below.
- 3. Disrupt tissue sample.** Tissue disruption promotes release of genomic DNA prior to sample spotting for dry-down. We recommend using one of the following procedures for optimal DNA release:
 - a. Tissue homogenizer.** Use a rotor-stator tissue homogenizer to fully disrupt the tissue fragment. We recommend keeping samples on ice and avoiding excessive homogenization.¹

¹ Over-homogenization can lead to shearing of genomic DNA. However, an advantage to this method is that it is quick and results in the highest yields.

OR

b. Proteinase K treatment.

- i. Recommended: Disrupt tissue fragment with a pestle. This facilitates lysis of the tissue by proteinase K and release of genomic DNA.
- ii. Add proteinase K to a final concentration of 0.75 mg/ml. Incubate sample at 56°C with shaking until sample is completely lysed. (Incubation time varies depending on the size and type of tissue, but generally complete lysis requires overnight incubation. To promote tissue lysis, use small, thinly sliced samples and/or grind the tissue with a pestle prior to incubation. Shaking during incubation is critical.)²

4. Optional: Pellet large tissue debris by spinning at 2000xg 1 minute. For maximal DNA yield, tissue debris can be dried in DNAgard.

5. Dry samples in a sterile laminar flow hood or in a SpeedVac. Note: complete drying is critical for sample stability. If using a SpeedVac, it is important to maintain the samples at room temperature (do not use heat when drying). (Refer to chart for drying times)

- a. **96-well microtiter plates (ref # 90028-290):** Spot up to 150 µl of DNAgard-tissue homogenate per well. Refer to chart for drying times.
- b. **48-well microtiter plates:** Spot up to 500 µl of DNAgard-tissue homogenate per well. Refer to chart for drying times.
- c. **12-well microtiter plates:** Spot a minimum of 500 µl of DNAgard-tissue homogenate per well. Refer to chart for drying times.
- d. **DNAgard-tubes (ref # 93027-290):** Spot up to 500 µl of DNAgard-tissue homogenate per tube (apply sample in the inner portion of the DNAgard-tube part containing an inner- and outer-chamber). Refer to chart for drying times.
- e. **Microfuge tubes:** Note: Because of the extensive time required to dry samples in microfuge tubes in a hood, use microfuge tubes only if drying via SpeedVac. Spot up to 500 µl of DNAgard-tissue homogenate per tube. Refer to chart for drying times. *Optional: microfuge tubes can be covered with a microporous plastic seal (not included) during dry-down in a SpeedVac. However, it is critical that*

² Please note that the processing time for this option is much longer than using the homogenizer, but there is less risk in shearing gDNA.

the seal allow rapid water vapor exchange (we recommend Breathe-EASIER Tube Membranes (from Excel Scientific)).

6. Store dried samples:

- a. Microtiter plates (ref # 90028-290):** Cover plates with a plastic lid. *(Optional: Plate can be covered with a microporous plastic sealing film (not included); however it is important that the seal allow gaseous water exchange. Water impermeable seals can trap residual water in the sample wells, potentially compromising sample integrity. We recommend the use of AeraSeal Sealing Films (from Excel Scientific)).* Store plate in a moisture-barrier foil bag with one 9 g desiccant packet and heat seal the bag. Alternatively, plates can be stored in a desiccant chamber. Samples should be kept at room temperature.
- b. DNAgard-tubes ref # 93027-290):** Samples stored in DNAgard-tubes can be covered with a plastic cap or with a microporous plastic sealing film.

Store dried samples in a moisture-barrier foil bag with one 9 g desiccant packet and heat seal the bag. Alternatively, tubes can be stored in a desiccant chamber. Samples should be kept at room temperature.

Use of a plastic sealing film is not necessary if care was taken to thoroughly dry the sample prior to storage. (If a sealing film is used, it is critical that it allow water vapor exchange. (we recommend Breathe-EASIER Tube Membranes (from Excel Scientific)).

- c. Microfuge tubes:** Samples stored in microfuge tubes can be capped or covered with a microporous plastic sealing film.

Store dried tubes in a moisture-barrier foil bag along with one 9 g desiccant packet and heat seal the bag. Alternatively, tubes can be stored in a desiccant chamber. Samples should be kept at room temperature.

Use of a plastic sealing film is not necessary if care was taken to thoroughly dry the sample prior to storage. (If a sealing film is used, it is critical that it allow water vapor exchange. We recommend (we recommend Breathe-EASIER Tube Membranes (from Excel Scientific)).

Storage of Tissue Culture Cells in DNAgard – Dry storage

1. Aliquot cells for shipment or storage.

Any microfuge tube can be used, however we recommend the use of a screw-cap tube to prevent sample leakage.

DNAgard has been shown to protect genomic DNA in sample concentrations ranging from 2×10^3 to 10^6 cells per 100 μ l DNAgard.

When choosing the number of cells to store, consider the desired DNA yield and the specifications of the DNA isolation procedure used to recover the genomic DNA. Refer to the manufacturer's specifications in the DNA isolation protocol for product limitations and expected yields (in general, approximately 200 – 600 ng of nucleic acid can be recovered from 10^5 cultured cells containing a normal set of chromosomes).

2. Pellet cells at 3500xg for 1-2 minutes in a microcentrifuge (volumes exceeding 1.5 ml may require longer spin times). Remove supernatant.

3. Add a minimum of 100 μ l of DNAgard solution for every 10^6 cells (preferably, use screw cap tubes to avoid spillage).

Pulse-vortex samples to completely resuspend pellet.

If the cell suspension in DNAgard is too viscous to pipet, then add more DNAgard solution. Avoid adding excessive amounts of DNAgard, as this will increase the volumes of reagents required for DNA recovery.

4. Dry samples in a sterile laminar flow hood or in a SpeedVac. Note: complete drying is critical for sample stability. If using a SpeedVac, it is important to maintain the samples at room temperature (do not use heat when drying). (Refer to chart for drying times)

a. **96-well microtiter plates (ref # 90028-290):** Spot up to 150 μ l of DNAgard-cell homogenate per well. Refer to chart for drying times.

b. **48-well microtiter plates:** Spot up to 500 μ l of DNAgard-cell homogenate per well. Refer to chart for drying times.

c. **12-well microtiter plates:** Spot a minimum of 500 μ l of DNAgard-cell homogenate per well. Refer to chart for drying times.

d. **DNAgard-tubes (ref # 93027-290):** Spot up to 500 μ l of DNAgard-cell homogenate per tube (apply sample in the inner portion of the DNAgard-tube part containing an inner- and outer-chamber). Refer to chart for drying times.

Microfuge tubes: Note: Because of the extensive time required to dry samples in microfuge tubes in a hood, use microfuge tubes only if drying via SpeedVac. Spot up to 500 µl of DNAgard-cell homogenate per tube. Refer to chart for drying times. *Optional: microfuge tubes can be covered with a microporous plastic seal (not included) during dry-down in a SpeedVac. However, it is critical that the seal allow rapid water vapor exchange (we recommend Breathe-EASIER Tube Membranes, from Excel Scientific).*

5. Store dried samples:

a. Microtiter plates (ref # 90028-290): Cover plates with a plastic lid. *(Optional: Plate can be covered with a microporous plastic sealing film (not included); however it is important that the seal allow gaseous water exchange. Water impermeable seals can trap residual water in the sample wells, potentially compromising sample integrity. We recommend the use of AeraSeal Sealing Films (from Excel Scientific)).* Store plate in a moisture-barrier foil bag with one 9 g desiccant packet and heat seal the bag. Alternatively, plates can be stored in a desiccant chamber. Samples should be kept at room temperature.

b. DNAgard-tubes (ref # 93027-290): Samples stored in DNAgard-tubes can be covered with a plastic cap or with a microporous plastic sealing film.

Store dried samples in a moisture-barrier foil bag with one 9 g desiccant packet and heat seal the bag. Alternatively, tubes can be stored in a desiccant chamber. Samples should be kept at room temperature.

Use of a plastic sealing film is not necessary if care was taken to thoroughly dry the sample prior to storage. (If a sealing film is used, it is critical that it allow water vapor exchange. We recommend Breathe-EASIER Tube Membranes)

c. Microfuge tubes: Samples stored in microfuge tubes can be capped or covered with a microporous plastic sealing film.

Store dried tubes in a moisture-barrier foil bag along with one 9 g desiccant packet and heat seal the bag. Alternatively, tubes can be stored in a desiccant chamber. Samples should be kept at room temperature.

Use of a plastic sealing film is not necessary if care was taken to thoroughly dry the sample prior to storage. (If a sealing film is used, it is critical that it allow water vapor exchange. We recommend Breathe-EASIER Tube Membranes from Excel Scientific).

Table 1. Approximate drying times in laminar flow hood

Sample volume (µl)	Approximate drying time (h)				
	Microfuge tubes	DNAgard tubes	12-well plates	48-well plates	96-well plates
100	NR ¹	7	ND ²	ND ²	12
150	NR ¹	ND ²	ND ²	ND ²	15
250	NR ¹	12	ND ²	20	NR ¹
500	NR ¹	20	ND ²	36-40	NR ¹

¹ NR = Not Recommended ; ² ND = Not Determined

Table 2. Approximate drying time in a SpeedVac[®] at low temperature (25-30 °C)

Sample volume (µl)	Approximate drying time (h)				
	Microfuge tubes	DNAgard tubes	12-well plates	48-well plates	96-well plates
100	1 – 2	ND ²	ND ²	ND ²	1 – 2
150	ND ²	ND ²	ND ²	ND ²	2
250	2.5 – 3	2	ND ²	2 – 3	NR ¹
500	3 – 4	2.5 – 3	ND ²	3 – 4	NR ¹

¹ NR = Not Recommended ; ² ND = Not Determined

Note: Drying times in the table above are only estimates. Depending on the relative humidity, drying time may vary significantly. Drying should occur at 15 - 30 °C with relative humidity below 50%. Please be aware that drying in a nonventilated incubator leads to elevated relative humidity. Relative humidity above 50% for extended periods of time will reduce product performance and sample protection.

SAMPLE RECOVERY

Sample recovery from tissue samples stored in DNAgard – dry storage

Note: recovery of DNA from tissue samples stored dry in DNAgard requires the use of a commercially available column purification technology. This storage method is not compatible with DNA isolation procedures involving phenol-chloroform extraction.

- *For dried samples that must be transferred to a microfuge tube for DNA isolation, we recommend following step 1 below (rehydration in water or buffer). (This applies to samples dried in microtiter plates or samples dried in multiple tubes and that are to be pooled for DNA isolation).*
- *For samples dried in a tube suitable for DNA isolation (e.g. microfuge tube) and therefore do not require transfer to another tube for DNA isolation, we recommend following step 2 below (rehydration in kit lysis buffer).*

The addition of kit lysis buffer to dried DNAgard samples often results in a flocculent precipitate that is not suitable for pipetting. Therefore, samples that need to be transferred to tubes suitable for DNA isolation must first be rehydrated in water or buffer and then transferred to the desired tube.

1. For dried samples that require transfer to a vessel suitable for DNA isolation: Rehydrate dried samples directly in water or buffer (e.g. PBS; TE (10 mM Tris-Cl, pH 8.0; 1 mM EDTA)) for at least 15 min. Follow steps for DNA isolation in section 1b or 1c under “Sample recovery from tissue samples stored in DNAgard – liquid storage” (above) or see Appendix B for a specific protocol using QIAGEN’s QIAamp DNA Mini Kit. When rehydrating dried samples in a tube or well, use a rehydration volume equal to, or greater than, the volume of sample that was initially spotted for drying. Pipette up and down to facilitate complete resuspension. Transfer resuspended sample to a tube of suitable volume for DNA isolation (we recommend the use of a screw-cap tube to avoid sample leakage).

An example of a modified protocol for DNA extraction from tissue stored **dry in DNAgard using the QIAGEN QIAamp DNA Mini Kit is provided in Appendix B. This protocol minimizes the reagent volumes required for DNA isolation.**

2. For dried samples that are in a vessel suitable for DNA isolation: Rehydrate dried samples directly in the lysis buffer from the DNA isolation kit for at least **15 minutes. To ensure recovery of the entire sample, rehydrate in a volume equal to, or greater than, the volume of sample that was initially spotted for drying.**

Pipette up and down to facilitate complete resuspension. Follow manufacturer’s instructions for DNA isolation.

Note: addition of lysis buffer to the dried sample will sometimes result in a turbid suspension or a flocculent precipitate. This is normal and will not affect DNA yield.

Sample recovery from tissue culture cells stored in DNAgard – dry storage

Note: recovery of DNA from cultured cell samples stored dry in DNAgard requires the use of a commercially available column purification technology. This storage method is not compatible with DNA isolation procedures involving phenol-chloroform extraction.

- *For dried samples that must be transferred to a microfuge tube for DNA isolation, we recommend following step 1 below (rehydration in water or buffer). (This applies to samples dried in microtiter plates or samples dried in multiple tubes and that are to be pooled for DNA isolation).*
- *For samples dried in a tube suitable for DNA isolation (e.g. microfuge tube) and therefore do not require transfer to another tube for DNA isolation, we recommend following step 2 below (rehydration in kit lysis buffer).*

The addition of kit lysis buffer to dried DNAgard samples often results in a flocculent precipitate that is not suitable for pipetting. Therefore, samples that need to be transferred to larger tubes to accommodate kit reagent volumes must first be rehydrated in water or buffer and then transferred to the larger tube. Kit lysis buffer can then be added.

1. For dried samples that require transfer to a larger vessel for DNA isolation: Rehydrate dried samples directly in water or buffer (e.g. PBS; TE (10 mM Tris-Cl, pH 8.0; 1 mM EDTA)) for at least 15 min. Follow steps for DNA isolation in section 1 or 2 under “Sample recovery from tissue culture cells stored in DNAgard – liquid storage” (above) or see Appendix D for specific instructions on using QIAGEN’s QIAamp DNA Mini Kit for genomic DNA isolation. When rehydrating dried samples in a tube or well, use a rehydration volume equal to, or greater than, the volume of sample that was initially spotted for drying. Pipette up and down to facilitate complete resuspension. Transfer resuspended sample to a tube of suitable volume for DNA isolation (we recommend the use of a screw-cap tube to avoid sample leakage).

A protocol for DNA extraction from cultured cells stored **dry** in DNAgard using the QIAGEN QIAamp DNA Mini Kit is provided in Appendix **D**.

2. For dried samples that are in a vessel suitable for DNA isolation: Rehydrate dried samples directly in the lysis buffer from the DNA isolation kit for at least 15 minutes. To ensure recovery of the entire sample, rehydrate in a volume equal to, or greater than, the volume of sample that was initially spotted for drying. Pipette up and down to facilitate complete resuspension. Follow manufacturer’s instructions for DNA isolation.

Note: addition of lysis buffer to the dried sample will sometimes result in a turbid suspension or a flocculent precipitate. This is normal and will not affect DNA yield.

Appendices

Appendix A: DNA extraction from tissue samples stored in liquid DNAGard – modified protocol for QIAamp DNA Mini Kit.

The following protocol has been proven compatible with DNAGard for maximum genomic DNA isolation. This protocol minimizes the reagent volumes required for DNA extraction. Addition of all reagents (prior to loading on the QIAamp column) is less than 1800 µl, so samples can be processed in 2 ml microfuge tubes.

The following protocol is for DNA recovery from a tissue sample stored in 500 µl DNAGard:

- 1. Recommended: Tissue disruption.** Tissue lysis can be facilitated by disruption with a sterile pestle.
- 2. Add 400 µl Buffer ATL.**
- 3. Add 40 µl proteinase K,** mix by vortexing and incubate at 56°C until the tissue is completely lysed. Sample lysis can be facilitated by using a shaking incubator and occasional vortexing.
- 4. Optional: Add 18 µl RNase A (100 mg/ml), mix and incubate for 2 min. at room temperature.**
- 5. Add 400 µl Buffer AL** and mix by pulse-vortexing for 15 sec. **Incubate at 70°C for 10 min.**
- 6. Add 400 µl ethanol (100%) and mix by pulse-vortexing for 15 sec.**
- (The remaining steps are the same as those indicated in the manual for DNA purification from tissues). **Apply up to 700 µl of the sample to the QIAamp Mini Spin column (in a 2 ml collection tube). Centrifuge at 6000 x g (8000 rpm) for 1 min.** Transfer the column to a clean collection tube and discard the tube containing the filtrate. *Qiagen recommends processing a maximum of 25 mg of tissue (10 mg for spleen) per column.*
- Repeat step 6 (as needed) to load entire sample on column.
- 9. Add 500 µl Buffer AW1 to the column. Centrifuge at 6000 x g (8000 rpm) for 1 min.** Transfer the column to a clean collection tube and discard the tube containing the filtrate.
- 10. Add 500 µl Buffer AW2 to the column. Centrifuge at maximum speed (20,000 x g ; 14,000 rpm) for 3 min.**
- 11. Recommended: Transfer the column to a clean collection tube and discard the tube containing the filtrate. Centrifuge at maximum speed for 1 min.**
- 12. Transfer the column to a clean 1.5 ml microfuge tube. Add 200 µl Buffer AE or water and incubate at room temperature for 1 min. Centrifuge at 6000 xg (8000 rpm) for 1 min.**

Appendix B: DNA extraction from tissue samples stored dry in DNAgard – modified protocol for QIAamp DNA Mini Kit.

The following protocol has been proven compatible with DNAgard for maximum genomic DNA isolation. This protocol is intended for use with dried samples that must be transferred to another vessel for DNA isolation. This protocol minimizes the reagent volumes required for DNA extraction. Addition of all reagents (prior to loading on the QIAamp column) is less than 1800 µl, so samples can be processed in 2 ml microfuge tubes.

The following protocol describes DNA recovery from a tissue sample dried in 500 µl DNAgard:

- 1. Rehydrate dried samples directly in 500 µl water or buffer (e.g. PBS; TE (10 mM Tris-Cl, pH 8.0; 1 mM EDTA)) for at least 15 min.** When rehydrating dried samples in a tube or well, use a rehydration volume equal to, or greater than, the volume of sample that was initially spotted for drying. Pipette up and down to facilitate complete resuspension. Transfer resuspended sample to a tube of suitable volume for DNA isolation (we recommend the use of a screw-cap tube to avoid sample leakage).
- 2. Add 400 µl Buffer ATL.**
- 3. Add 40 µl proteinase K,** mix by vortexing and incubate at 56°C until the tissue is completely lysed. Sample lysis can be facilitated by using a shaking incubator and occasional vortexing.
- 4. Optional: Add 18 µl RNase A (100 mg/ml), mix and incubate for 2 min. at room temperature.**
- 5. Add 400 µl Buffer AL** and mix by pulse-vortexing for 15 sec. **Incubate at 70°C for 10 min.**
- 6. Add 400 µl ethanol (100%) and mix by pulse-vortexing for 15 sec.**
- 7. (The remaining steps are the same as those indicated in the manual for DNA purification from tissues). Apply up to 700 µl of the sample to the QIAamp Mini Spin column (in a 2 ml collection tube). Centrifuge at 6000 x g (8000 rpm) for 1 min.** Transfer the column to a clean collection tube and discard the tube containing the filtrate. *Qiagen recommends processing a maximum of 25 mg of tissue (10 mg for spleen) per column.*
- 8. Repeat step 7 (as needed) to load entire sample on column.**
- 9. Add 500 µl Buffer AW1 to the column.** Centrifuge at 6000 x g (8000 rpm) for 1 min. Transfer the column to a clean collection tube and discard the tube containing the filtrate.
- 10. Add 500 µl Buffer AW2 to the column.** Centrifuge at maximum speed (20,000 x g ; 14,000 rpm) for 3 min.
- 11. Recommended:** Transfer the column to a clean collection tube and discard the tube containing the filtrate. Centrifuge at maximum speed for 1 min.

- 12.** Transfer the column to a clean 1.5 ml microfuge tube. Add 200 μ l Buffer AE or water and incubate at room temperature for 1 min. Centrifuge at 6000 xg (8000 rpm) for 1 min.

Appendix C: DNA extraction from mammalian tissue culture cells stored in liquid DNAGard – modified protocol for GE Illustra Tissue and Cells Genomic Prep Mini Spin Kit.

- 1. Determine the amount of DNAGard that was aliquoted to store the cells. Add the same volume of Lysis buffer type 1 to the sample containing DNAGard. For example, a sample of cells in 100ul DNAGard should receive 100ul of Lysis buffer type 1. Mix well by pipetting up and down or vortexing for 15 seconds.**
- 2. Add 10ul proteinase K (20mg/ml) to the samples. Mix by vortexing for 15 seconds.**
- 3. Incubate samples at 56 °C for 15 minutes. Transfer samples to 70 °C and incubate for 2 minutes. During this step, preheat an aliquot (200ul per sample) of Elution buffer type 5 at 70 °C. The buffer will be used for elution during the last step.**
- 4. Add 5ul RNase A (20mg/ml). Incubate at room temperature for 15 minutes.**
- 5. Add 1000ul Lysis buffer type 4. Incubate at room temperature for 10 minutes.**
- 6. Apply a portion of the sample to column. The capacity of the column is 720ul. Spin sample at 11,000 x g for 1 minute. Discard flowthrough and apply the rest of the sample to the column. Spin again at 11,000 x g for 1 minute.**
- 7. Wash by adding 500ul Lysis buffer type 4 to the column. Spin at 11,000 x g for 1 minute.**
- 8. Add 500ul Wash buffer type 6. Spin at 11,000 x g for 3 minutes.**
- 9. Before proceeding, make sure that the column is completely dry. Perform an additional spin if necessary.**
- 10. Transfer column to a fresh sample collection tube. Add 200ul of preheated Elution buffer type 5 (from step 3) to the column. Incubate for 1 minute at room temperature. Spin at 11,000 x g for 1 minute to elute DNA.**

Appendix D: DNA extraction from mammalian tissue culture cells stored dry in DNAgard –protocol for QIAamp DNA Mini Kit.

The following protocol has been proven compatible with DNAgard for maximum genomic DNA isolation. This protocol is intended for use with dried samples that must be transferred to another vessel for DNA isolation.

The following protocol describes DNA recovery from a tissue culture cell sample dried in 100 µl DNAgard:

- 1. Rehydrate dried samples directly in 100 µl water or buffer (e.g. PBS; TE (10 mM Tris-Cl, pH 8.0; 1 mM EDTA)) for at least 15 min.** When rehydrating dried samples in a tube or well, use a rehydration volume equal to, or greater than, the volume of sample that was initially spotted for drying. Pipette up and down to facilitate complete resuspension. Transfer resuspended sample to a tube of suitable volume for DNA isolation (we recommend the use of a screw-cap tube to avoid sample leakage).
- 2. Add 20 µl QIAGEN protease or proteinase K.**
- 3. Add 200 µl Buffer AL and mix by pulse-vortexing for 15 sec.**
- 4. Incubate at 56°C for 10 min.**
- 5. Add 200 µl ethanol (100%).**
- 6. Apply up to 700 µl of the sample to the QIAamp Mini Spin column (in a 2 ml collection tube).** Centrifuge at 6000 x g (8000 rpm) for 1 min. Transfer the column to a clean collection tube and discard the tube containing the filtrate.
- 7. Repeat step 6 (as needed) to load entire sample on column.**
- 8. Add 500 µl Buffer AW1 to the column.** Centrifuge at 6000 x g (8000 rpm) for 1 min. Transfer the column to a clean collection tube and discard the tube containing the filtrate.
- 9. Add 500 µl Buffer AW2 to the column. Centrifuge at maximum speed (20,000 x g ; 14,000 rpm) for 3 min.**
- 10. Recommended:** Transfer the column to a clean collection tube and discard the tube containing the filtrate. Centrifuge at maximum speed for 1 min.
- 11. Transfer the column to a clean 1.5 ml microfuge tube. Add 200 µl Buffer AE or water and incubate at room temperature for 1 min. Centrifuge at 6000 xg (8000 rpm) for 1 min.**

Notes

Technical Assistance

Biomatrix, Inc. takes pride in providing efficient quality technical support. Biomatrix's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of Biomatrix's biostability and storage products. Please contact Biomatrix directly with any questions regarding DNAstable technology, product use, or general matters.

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