

GenTegra-DNA dry BULK User Guide

GTD100-B



Version D

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For Research Use Only



GenTegra LLC

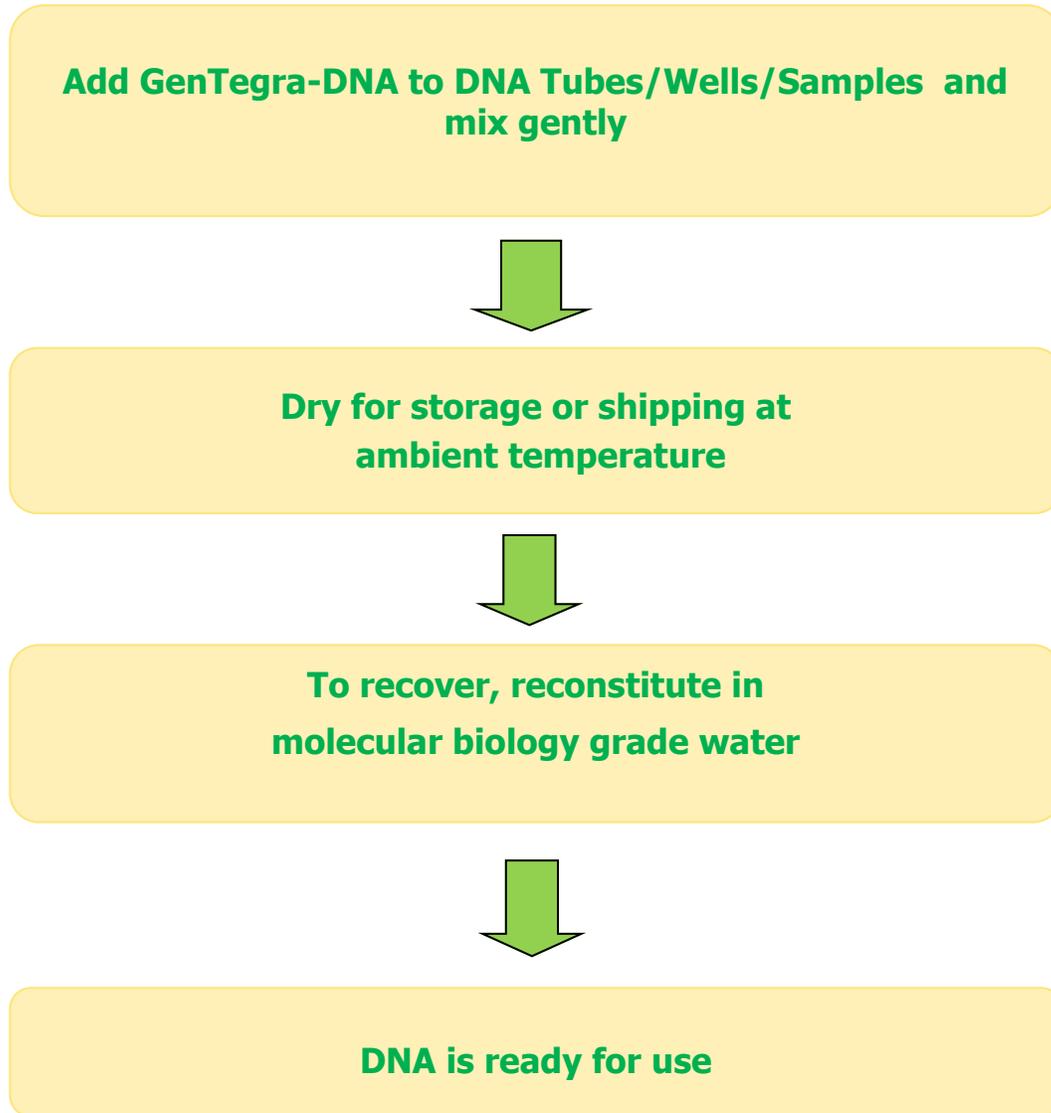
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Simplified Workflow



Overview

GenTegra™-DNA is a novel technology for storage and transport of DNA in ready to use aliquots. GenTegra DNA allows storage of DNA in a water-free environment, which protects samples from hydrolysis, oxidation and microbial growth. Simply add purified DNA, mix gently, dry, and store at room temperature. When needed, simply rehydrate and the DNA sample is ready for downstream analysis. GenTegra-DNA is well suited for ambient temperature shipping locally, nationally and internationally, tolerating the rigorous United States Military ambient shipping specifications of -80°F (-62°C) to 160° F (71°C). In addition to standard GenTegra tubes and microplates, GenTegra-DNA is available in bulk form for custom applications:

- 1x concentration – For making custom tubes for stabilizing and storing purified samples.
- 5x concentration – For adding directly to liquid samples of purified DNA, followed by gentle mixing and drying.

Product Information

	GenTegra-DNA Dry Bulk
Catalog Number	GTD100-B
Product form	Dried material in 2 ml vial
Sample Volume	20-245 µl
Sample Amount	0.05-25 µg of DNA
DNA concentration	Any
Recovery Volume	Equivalent to Sample volume
1X for 100 custom tubes	Add 1.65 ml molecular biology grade water, mix gently to dissolve
Amount per tube	15 µl per tube
5X for addition to 100 samples	Add 0.55 ml molecular biology grade water
Amount per sample	Add 5 µl to each sample
For Forensic samples 40-75uL	Add 1.65ml molecular biology grade water, mix gently to dissolve
Amount per sample	Add 5 µl to each sample
Drying Method	SpeedVac, Vacuum Desiccator, FastDryer™, Biosafety Hood

Upon arrival, GenTegra-DNA dry Bulk is a plastic like material in the bottom of the vial. In the dry form GenTegra-DNA has a shelf life estimated to be at least three years. When re-hydrated the solution should be stored at 4°C and used within 3 months.

Product Information, cont'd

Expected Results

- Quantitative recovery of DNA
- Quality is comparable to input DNA

Storage and Transport

- Quantitative recovery of DNA
- Quality is comparable to input DNA

Transport conditions: -80°C to +56°C

Storage conditions: 15°C to 30°C

Tested Storage Buffers Compatible with GenTegra-DNA

- Qiagen Buffer AE
- TE, pH 7.5 and TE pH 8.0 (10mM Tris and 1 mM EDTA)
- Low EDTA TE, pH 8.0 (10mM Tris and 0.1 mM EDTA)

Tested Applications Compatible with GenTegra DNA

The following applications have been tested to be compatible with DNA recovered from GenTegra DNA tubes:

- Gene Expression Analysis
- Genotyping
- Sequencing
- HLA Typing
- STR for HID, validated

GenTegra DNA 1X Protocols

The 1X concentration liquid can be applied to tubes or microplate wells. After air drying, it forms a GenTegra matrix coating at the bottom, which can then be stored dry until needed. Purified DNA in solution can then be added to these matrix-coated tubes or plates, to be followed by air-drying and storage.

Preparing 1X GenTegra DNA Tubes and Microplates

1. Add 1.65 ml of water to the GenTegra-DNA bulk tube and dissolve with occasional gentle vortexing for 5-10 minutes. If left standing the GenTegra will dissolve in approximately 30 minutes and is ready after gentle vortexing.
2. Aliquot 15 μ l of GenTegra-DNA into user-supplied tubes or microplate.
2. Dry by any of these methods:
 - In a SpeedVac at ambient temperature or 30°C till dry
 - In a vacuum desiccator for ~3-4 hours
 - In an GenTegra FastDryer overnight ~16 hours
 - In a Biosafety Hood overnight ~16 hours

If the tubes do not dry overnight, then additional effort should be taken to reduce humidity and drying time to less than 24 hours. Do not use excessive heat to accelerate the drying process. We do not recommend warming at higher than 50C to dry the matrix. For details on drying GenTegra, see "Drying and Storing GenTegra" on page 7.

Applying DNA Samples to Stabilized Storage Containers

1. Aliquot DNA samples into the prepared GenTegra tubes or microplate. The typical containers used are 0.3 ml, 1.7 ml and 0.5 ml tubes, and 96-well microplates.
2. Mix gently by pipetting up and down 6-10 times to solubilize the GenTegra Matrix. Do not vortex the DNA solution as vortexing can cause mechanical shearing of the DNA.
3. Proceed to the protocol "Drying and Storing GenTegra" on page 7. After the samples are dry, they are stable for long term ambient storage.

GenTegra DNA 5X Protocol – Adding to Samples

The 5X concentration liquid is added directly to liquid purified DNA samples. After gentle mixing, the solution can be dried and is stable for long term storage.

1. Add 0.55 ml of water to the GenTegra-DNA Dry Bulk tube and dissolve using gentle mixing for 5-10 minutes.
2. Add 5 μ l of GenTegra-DNA 5x solution to each isolated DNA sample.

The DNA sample amounts that may be used:

- Volume: 20-245 μ l
 - Amount: 0.05-25 μ g
2. Mix thoroughly and gently to disperse the GenTegra Matrix and avoid foaming. If any foaming or bubbles occur during mixing, briefly centrifuge the tubes to remove bubbles and place solution in the bottom of the tube.
 3. Quickly centrifuge to bring the matrix and sample to the tube/well bottom and remove any foaming or bubbles.
 4. Dry by any of these methods:
 - In a SpeedVac at ambient temperature or 30°C till dry
 - In a Vacuum desiccator till dry, see below
 - In a GenTegra FastDryer overnight or till dry
 - In a Biosafety Hood overnight or till dry

If the tubes do not dry overnight, then additional effort should be taken to reduce humidity and drying time to less than 24 hours. Do not use heat to accelerate the drying process. After the samples are dry, they are completely stable for long term ambient storage.

Drying and Storing GenTegra

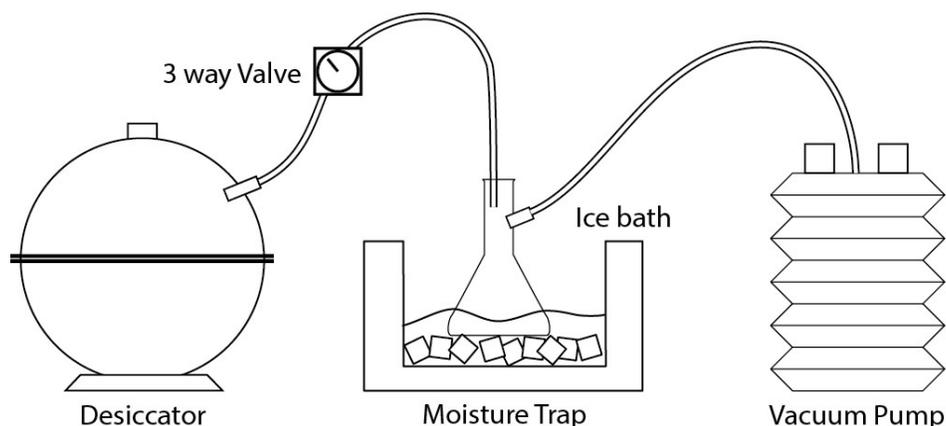
DNA samples are typically dried overnight. After the samples are dry, they are completely stable for long term ambient storage.

- For 1.5 ml screw cap tubes in a FastDryer, the volume must be $\leq 50 \mu\text{l}$.
- Drying times for SpeedVac and biosafety hood vary depending on the sample volume.
- When using a SpeedVac or biosafety hood, ensure that DNA is completely dry prior to storage.
- Use SpeedVac on room temperature setting (no additional heat).
- Drying times for 96-well microplates in a biosafety hood are approximately:
 $\leq 50 \mu\text{l}$ - 24 hr; $\leq 100 \mu\text{l}$ - 48hr; $\leq 250 \mu\text{l}$ - 72hr

Drying DNA Using a GenTegra GVT2001 FastDryer

A FastDryer can dry up to 50 μl of DNA in 16 hours. For further details, refer to the *FastDryer User Guide*, available for download at www.GenTegra.com.

1. Ensure that the FastDryer power cord is plugged in.
2. Open the FastDryer.
3. Ensure that the tube holder is inserted in the FastDryer. The tube holder is removable for cleaning.
4. Place unsealed or uncapped tubes or rack in the tube/rack holder as follows:
 - Place rack of tubes (with caps off) or unsealed microplate on top of the tube holder, or
 - Place up to 48 screw-cap tubes (with caps off) in the holes of the tube holder.
5. Close the FastDryer lid.
6. Press the red ON/OFF switch to turn on the FastDryer. Blue lights will illuminate when the FastDryer is operating.
7. Leave on for approximately 16 hours (or overnight) to dry the samples.
8. When drying is complete, turn off the FastDryer, remove the samples and cap or seal the tubes/plates for storage or transport.
9. Store the samples at room temperature (21–25 °C).



Multiple Drying and Rehydration of DNA

Following recovery, an aliquot of DNA may be removed for use, and the sample dried again. This procedure may be repeated multiple times until a maximum of 75% of the original sample (and thus, GenTegra chemical matrix) is removed.

For example, a 200 μl sample is applied to a GenTegra tube, dried and rehydrated. Following rehydration, 50 μl is removed for analysis, leaving 150 μl (75% of the original sample), which is dried again. This process can be repeated until removal of an aliquot for analysis causes the volume of the sample to drop below 50 μl (25% of the original sample), in which case it should be stored according to typical conditions (for example, at $-20\text{ }^{\circ}\text{C}$). These calculations assume that the sample was always rehydrated at the same concentration.

This calculation is based on percentage of matrix remaining in the solution and not absolute volume. Thus, a sample starting at a volume of 100 μl could undergo drying and rehydration until the volume drops below 25 μl (25% of the original sample).

DNA Sample Recovery

The recovery volume is the same as the starting volume. Be sure to keep a record of the initial sample volume.

1. Apply a volume of molecular biology grade water equivalent to the input sample volume. For details, see the section "Product Information."
Ensure that the final concentration of DNA is $\leq 250\text{ ng}/\mu\text{l}$.
2. Incubate at room temperature ($21\text{--}25^{\circ}\text{C}$) for 15 minutes.
3. Mix very gently to solubilize the DNA. Do not vortex.
 - For commonly used tubes, cap the tubes and gently agitate for 1 minute.
 - For 96-well microplates, pipette up and down gently 10 times.

Typical recovery volume is 35–250 μl , and concentration is 200ng/L. The DNA is ready for use in downstream applications.

Note: Vortexing a DNA solution can cause mechanical shearing of the DNA. Mix DNA solutions very gently.

Forensic DNA sample recovery and concentration adjustments

Forensic DNA samples are by their nature of smaller volumes and amounts than research samples. To prevent the concentration of the GenTegraDNA matrix exceeding its recommended concentration the starting amount of matrix is adjusted for forensic samples.

1. For forensic samples add the volume of water listed in the table on page 4, row 11.
2. The normal amounts of DNA encountered for forensic samples will not exceed the protecting ability of the recommended GenTegraDNA solution.
3. When the GenTegraDNA is rehydrated to the volume recommended in the table on page 4, row 11, the solution will also readily support concentration of the sample upon rehydration with a smaller than original sample volume.
 - For a two fold increase in concentration add $1/2$ the original sample volume.
 - For a four fold increase in concentration add $1/4$ the original sample volume.
 - Etc.
4. Expected recovery of the DNA will be $>95\%$ of the original starting sample amount.

Technical Information

qPCR

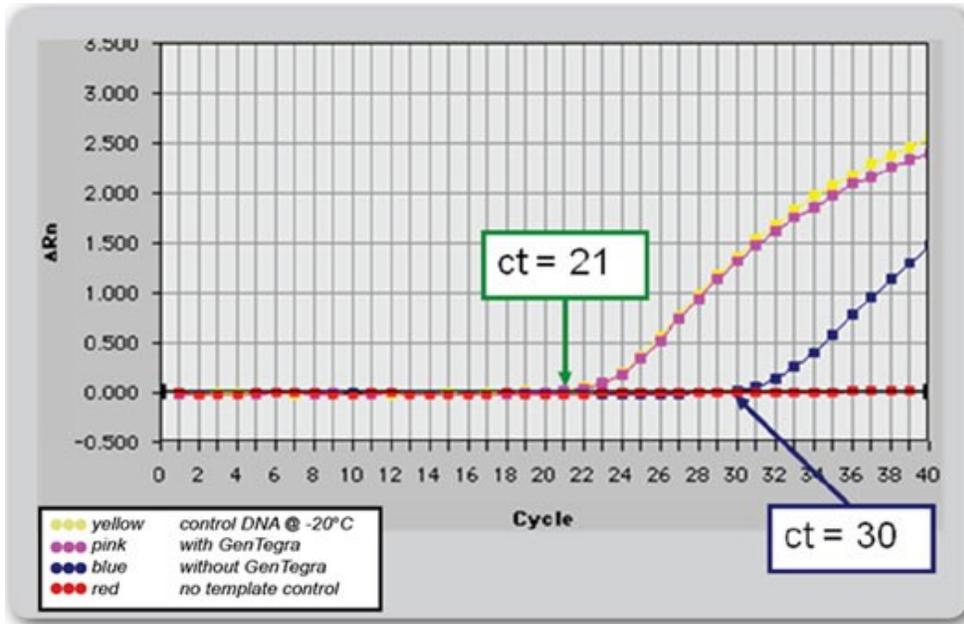


Figure 3. Successful qPCR amplification of DNA stored in GenTegra-DNA Tubes. Following recovery of DNA after storage at 76°C for two weeks with GenTegra-DNA, no PCR inhibition was observed even when 26% of the reaction volume was made up of DNA. The green box indicates Ct value of control DNA stored at -20°C and 50ng samples stored at 76°C in the presence of GenTegra-DNA. The blue box indicates shifted ct values of 50ng samples after storage at 76°C without GenTegra-DNA

Long Term Protection and Stability

DNA samples stored on GenTegra DNA show no degradation after the equivalent of 16 years storage at ambient temperature. Accelerated stability studies show DNA sample protection with no visible degradation.¹

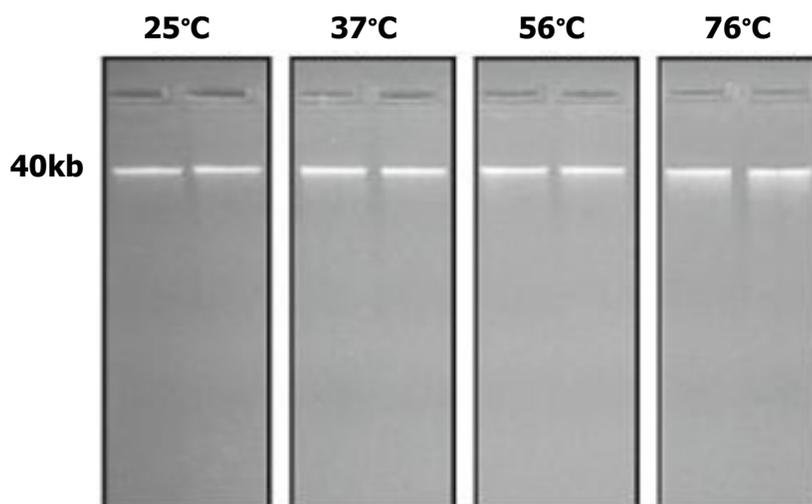


Figure 4. 250 ng/lane genomic DNA stored on GenTegra DNA for six months at ambient (25°C) and elevated temperatures.

DNA Genotyping

Table: Successful genotyping of DNA stored in GenTegra-DNA Tubes via Illumina and Affymetrix platforms.

		Control (-20°C)	GenTegra-DNA (26°C)
Call Rate	Affymetrix 6.0	99.50%	99.40%
	Infinium IM	99.82%	99.70%
Concordance with frozen control	Affymetrix 6.0		99.80%
	Infinium IM		99.70%

Results using Illumina Infinium IM and Affymetrix 6.0 are identical for DNA stored at -20°C and DNA stored in -20°C and DNA stored in GenTegra-DNA Tubes at room temperature.

DNA Recovery – Quantity and Quality

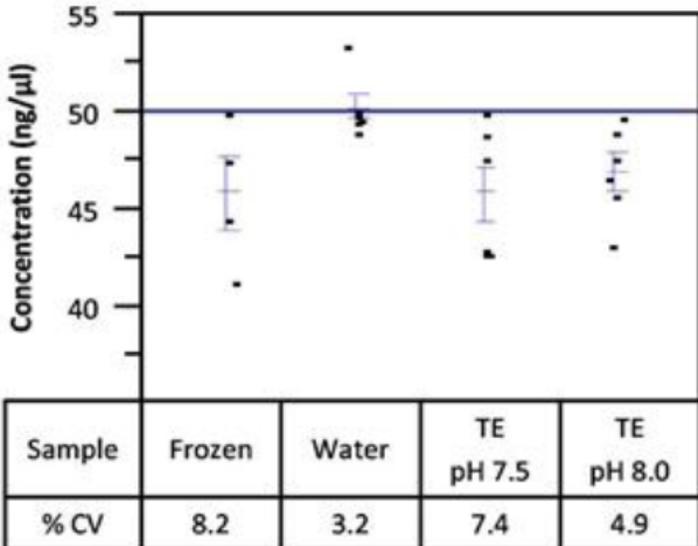


Figure 1. DNA is quantitatively recovered from GenTegra-DNA Tubes.

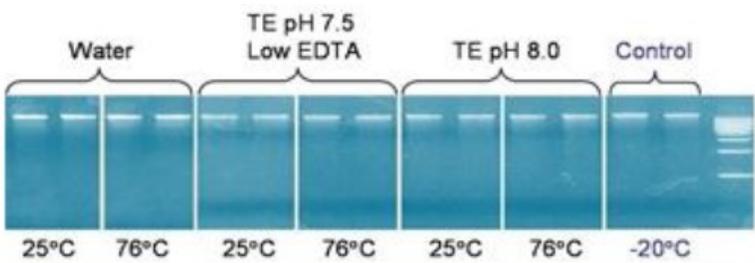


Figure 2. Quality and integrity of DNA stored in GenTegra-DNA Tubes is identical to DNA stored at -20°C. DNA was stored for 120 days at room temperature (25°C) or 76°C. 120 days of storage at 76°C is equivalent to 10 years of room temperature storage.

Sample volumes of 20 μ l or less

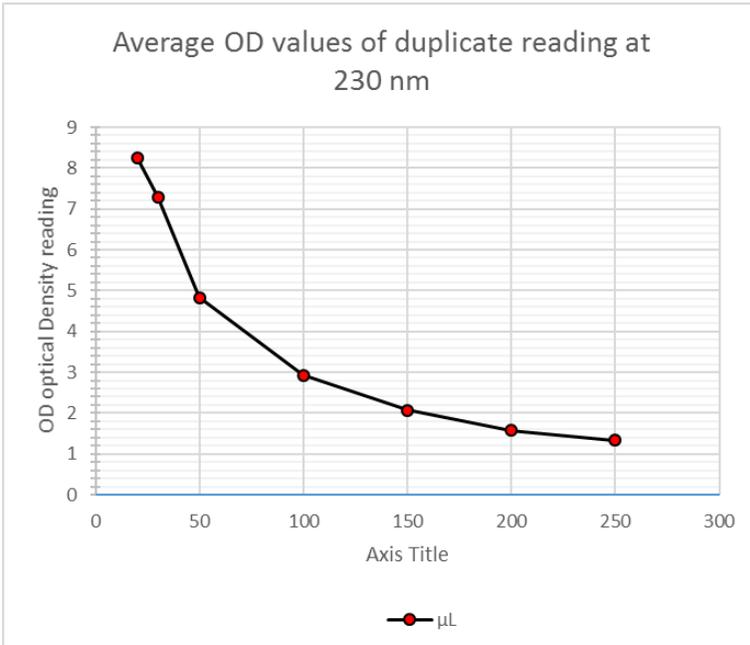
GenTegra-DNA tubes, microplates and Cluster tubes all start with 21 μ l of GenTegra-DNA solution being added to the bottom of the tube or well followed by drying. This means the 21 μ l coats the bottom and side walls of each tube/well to the height of 21 μ l. If the sample volume being used is less than 20 μ l it is unlikely that all the GenTegra-DNA will be dissolved by the sample and these small volumes will make it difficult to wet the sides of the tube to dissolve all the GenTegra-DNA. For volumes 10 μ l or less this can be an issue.

Small volumes will also tend to stick to the sides of the tube and may not even be in the bottom of the tube when they dry. This means that when the same small volume of water is then used to rehydration the sample it is possible that the rehydration volume may not be in the same place as the original sample. This can lead to apparent sample loss even if the sample is in fact in the tube. Vortexing these small sample volumes can also lead to apparent sample loss as the sample disappears as a coating throughout the inside of the tube. Brief centrifugation may help return the sample to the bottom of the tube but may still lead to losses due to coating of the tube surface.

If small sample volumes are to be used it is recommended that the rehydration volume used be at least 20 μ l to ensure all the original sample is recovered. This dilution of the original sample will not negatively impact the downstream analysis and are likely to improve the actual sample recovery. The 20 μ l low volume cut off is not because GenTegra-DNA cannot protect small samples but because these small samples are difficult to process conveniently.

Correcting 260/230 ratios

The GenTegra-DNA chemistry has an absorbance at 230 nm. This absorbance will cause the 260/230 nm ratio values to be different than will normally be expected. The following chart shows the plot of the sample volume vs. OD reading for the GenTegra-DNA solutions at differing volumes and the table below shows the numerical values.



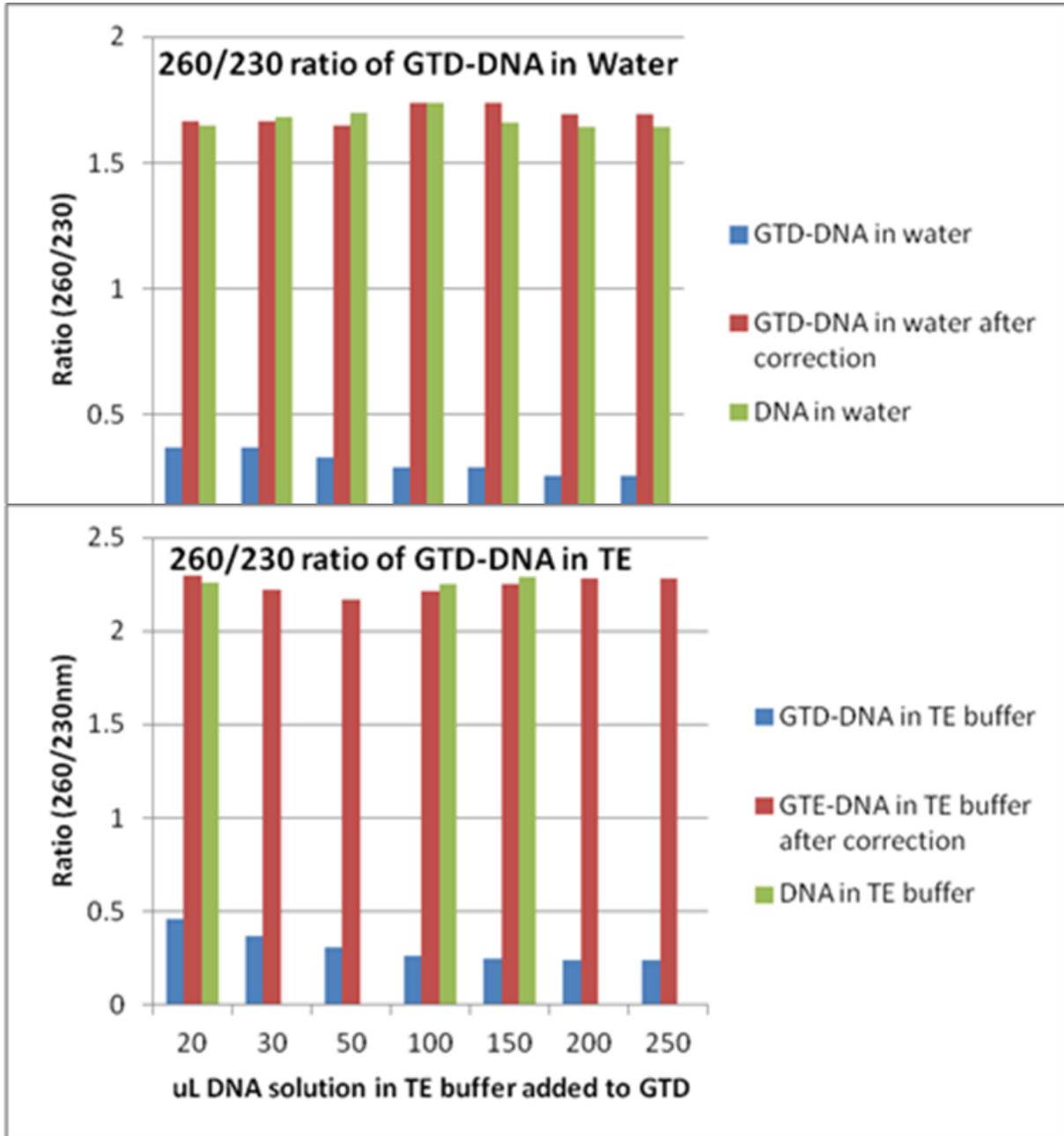
µL	OD
250	1.33
200	1.57
150	2.07
100	2.93
50	4.83
30	7.30
20	8.26

With these values it is possible to create a table of correction values that can be applied to the 260/230 ratios determined using a NanoDrop for example. The absorbance ratio is also affected if TE buffer is being used so a second column is given for the correction factor to use if the DNA & GenTegra solution is in TE buffer.

Simply multiply the 260/230 reading you get by the appropriate correction factor.

uL DNA added to GTD	Correction Factor	
	Water	TE
20	4.5	5
30	4.5	6
40	4.5	6
50	5	7
100	6	8.5
150	6	9
200	6.5	9.5
250	6.5	9.5

The following graphs compare 260/230 nm ratios for GenTegra-DNA plus DNA in water and in TE buffer and the difference with and without correction



Frequently Asked Questions (FAQ)

What is GenTegra®? Is GenTegra composed of a filter, beads or paper?

GenTegra-DNA is not a filter, beads or paper. GenTegra-DNA is an inert chemical matrix.

The GenTegra-DNA dry Bulk tubes appear to be empty. Where is the GenTegra-DNA and how can I detect it?

The GenTegra-DNA is supplied as a transparent coating at the bottom of each GenTegra-DNA Tube or well. To confirm that the kit you received contains the GenTegra-DNA, simply rehydrate one tube with 35µL of molecular biology grade water and take an absorbance reading at 230nm to detect the GenTegra-DNA.

Can samples stored in low-EDTA TE, water or other buffers be applied to GenTegra-DNA Tubes?

Yes, refer to Table 2 for a list of storage solutions that are compatible with GenTegra-DNA Tubes.

What is the maximum concentration of DNA that can be applied to GenTegra™ DNA Tubes?

There is no maximum concentration for application (note that the maximum concentration for recovery is 200 ng/µl) When applying less than 20 µL of DNA, add water to a final volume of ≥ 20 µl to ensure complete mixing of the DNA with the GenTegra-DNA. Refer to the tables on pages 14-17 for application volume and mass specifications.

Why is there a minimum recovery volume of 20 µl?

A minimum 20 µl volume is recommended to rehydrate DNA from all surfaces of the tube or microplate well.

Why is there a maximum recovery concentration of 200ng/µL when recovering or concentrating DNA?

Maximum solubility of DNA in water is achieved when the concentration does not exceed 200 ng/µl.

What is the composition of the storage solution after recovery?

After addition of molecular biology water, your samples will be in the same buffer they were stored in at the time of application.

Will the GenTegra-DNA affect my DNA quantitation? Do I need to blank the spectrophotometer with the GenTegra-DNA?

The GenTegra-DNA absorbs at 230 nm. Thus, it will not interfere with readings at Å260 or Å280 and blanking with the GenTegra-DNA is not required.

Frequently Asked Questions (FAQ) cont'd

How should I store my recovered DNA?

If the recovered DNA is in GenTegra-DNA we recommend re-drying the DNA solution and storing it at ambient temperature.

Can I use the recovered DNA directly for downstream applications?

Purification is **not** required prior to performing downstream applications. Similar DNA quality is maintained before and after recovery. GenTegra-DNA does not remove nucleases or other contaminants present in the original sample. When concentrating DNA, please be aware that contaminants will be concentrated along with the DNA.

Can I use the 5X bulk to make customer tubes?

We recommend using the 1X concentration so a larger surface area at the bottom of the tube is coated with GenTegra-DNA but it is just as acceptable to deposit 5 μ l of 5X in the bottom of the tube.

My DNA recovery upon rehydration seems low, what could cause this?

When rehydrating a sample with a smaller volume of water than the original sample volume it is possible for some DNA to be left on the sides of the tube. As the original sample dries it may leave a thin layer of DNA on the walls of the tube as high as the original sample volume. When rehydrating with a smaller volume this DNA on the walls may be missed.

For additional questions, contact GenTegra Technical Support at: support@GenTegra.com



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