GenTegraDNA™

GTD2100-S, GTD2025-S etc.

User Guide

Ambient temperature storage and transport of purified DNA

Version 02 November 2022

For Research Use Only

GenTegra LLC

1061 Serpentine Lane, Suite B, Pleasanton, CA 94566, USA

Table of Contents

FOR RESEARCH USE ONLY

Product Specifications

- 100% recovery of input DNA from 0.05 - 25 µg
- Quality is comparable to input DNA
- Recovery in a volume of 20 - 250 µL
- Increased stability in liquid state for up to 100 hours at room temperature (21-25°C) upon application
- Increased stability in liquid state for up to 8 hours following rehydration of dried DNA, across up to 5 cycles
- Compatible with DNA from cell lines, blood, fresh and frozen tissue, FFPE tissue, cDNA, plasmids, etc., single and double stranded
- Compatible with DNA purified using all standard kits and protocols (Invitrogen, Ambion, QIAGEN)
- Compatible with all common storage buffers, including water, TE, EDTA and citrate
- Use in downstream applications without further purification
    - Does not inhibit PCR, qPCR or expression profiling
- Thermal stability from -80°C to 76°C during transport
    - Exceeds Military specifications for transport (-60°C to 71°C)
    - Exceeds FedEx specifications for transport (-51°C to 60°C)

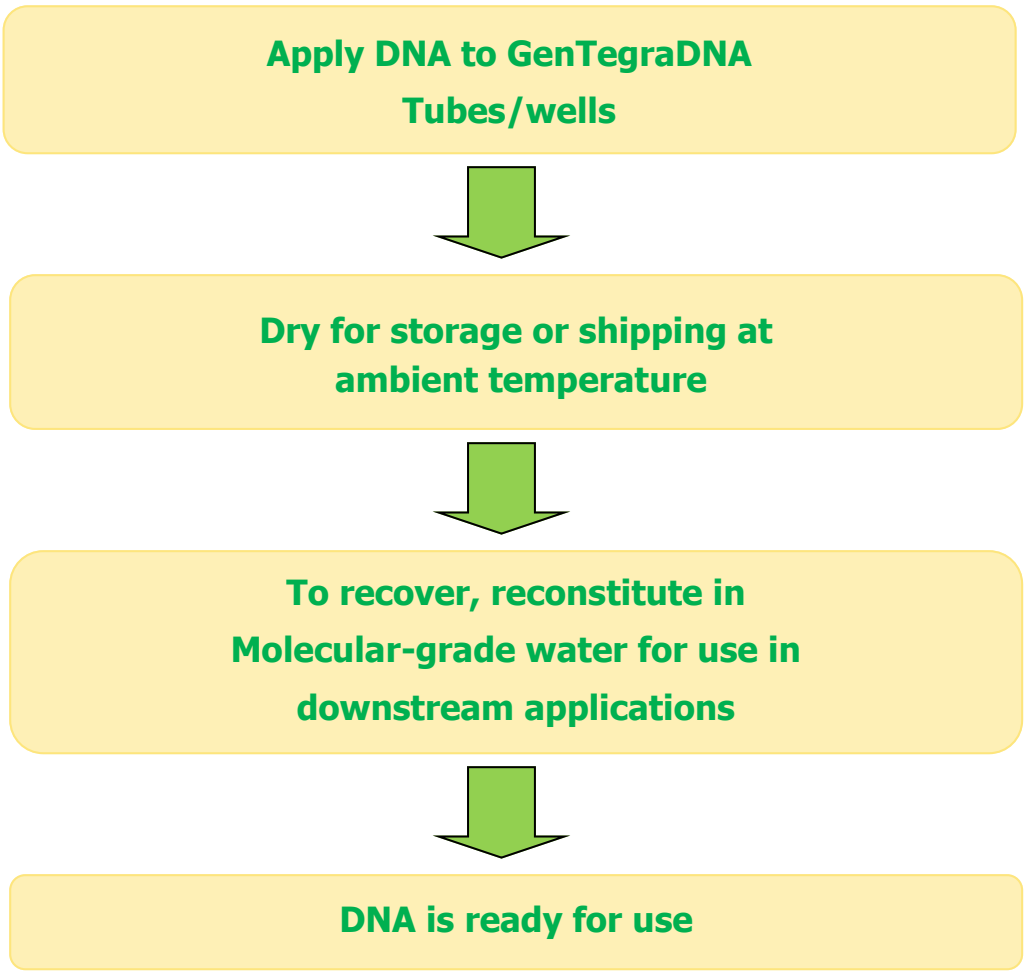
Storage and Transport

- Store and transport at ambient temperature

Tested storage buffers compatible with GenTegraDNA

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

Simplified Workflow



GenTegraDNA Protocol

DNA Application

1. Apply up to 25 µg of DNA in 20-250 µl to GenTegraDNA tube or well. For concentrated samples add water to a final volume ≤ 250 µl.

1. Mix by pipetting up and down 10 times to solubilize the GenTegraDNA.

   GenTegraDNA is supplied as a transparent coating at the bottom of each GenTegraDNA Tube or well.

3. Proceed to Drying Protocol (Page 6).

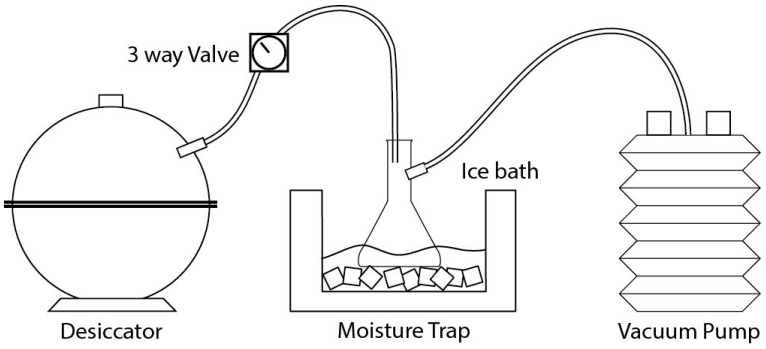
GenTegraDNA Protocol

Drying and Storage of DNA

1. Dry DNA according to the methods described in the table below.
   - Drying times will vary depending on application volume.
   - Whatever the drying method, ensure that DNA is completely dry prior to storage.
   - Use SpeedVac on room temperature setting (no additional heat).
   - Drying times for biosafety hood are approximate.

   When using 0.5ml screw cap tubes in a FastDryer, volume must be ≤50 µl.
2. When drying is complete, cap or seal tubes/plates and store at room temperature (21-25°C).

Volume	FastDryer	Vacuum Desiccator or SpeedVac	Biosafety Hood
≤50 µL	**16 hours**	**1-4 hours**	**24 hours**
≤100 µL	**32 hours**	**2-8 hours**	**48 hours**
≤250 µL	**48 hours**	**4-12 hours**	**72 hours**



GenTegraDNA Protocol

DNA Recovery

1. Apply a volume of molecular biology-grade water **equivalent to the input volume**.
2. Mix to solubilize the DNA according to the guidelines in the table below.

   Ensure that the final concentration of DNA is **≤200 ng/µl**.
3. Incubate at room temperature (21-25°C) for 15 minutes.
4. Sample is now ready for use.

Product	Recovery Volume and Concentration	Solubilization
0.5 ml Cluster Tubes	**20-250 µl* ≤200 ng/µl**	**Cap tubes and vortex for 1 minute**
96 Well Microplate		**Pipette up and down 10 times**
0.5 ml Screw Cap Tubes		**Cap tubes and vortex for 1 minute**

* For sample volumes less than 20 µl see page 13 for special handling information.

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 five (5) times. This assumes that each rehydration is down at the volume the sample was at the last drying.

For example, a 200 µl sample is applied to a GenTegra tube, dried and rehydrated with 200 µl of water. If 50 µl is removed for analysis, leaving 150 µl which is dried again. When this sample is rehydration it will be done using 150 µl of water. This process can be repeated up to five times with each rehydration volume reduced by the volume of the sample removed. This keeps the concentration of GenTegraDNA per µl as in the original sample.

When the remaining volume is 20 µl or less special care should be taken in retrieving the remaining sample.

Product Information

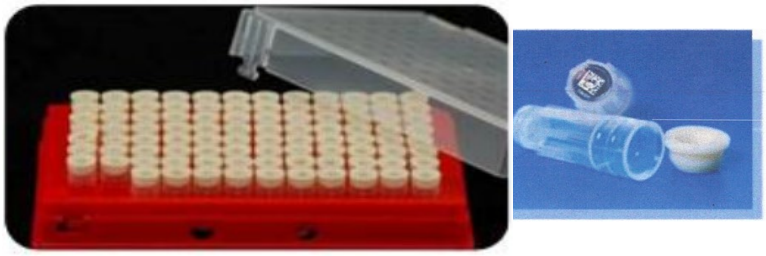
GenTegraDNA 0.5mL Screw-cap microtubes	
Catalog #	GTD2025-S (Trial kit), GTD2100-S, GTD2003-S Evaluation kit
Tube Volume	0.5 ml
Application Volume	20-250 µl
Application Volume	1-20 µl require special handling
Application Amount	≤ 25 µg
Concentration (DNA application)	Any
Recovery Volume	Equivalent to application vloume
Concentration (DNA recovery)	≤200 ng/µl
Drying Method	FastDryer Biological hood (≤50 µl volume) Vacuum (≤250 µl volume), SpeedVac at ambient

Product Information

GenTegraDNA 96-well Microplate	
Catalog #	**GTD4001-P, GTD4010-P, GTD4010-PBC**
Application Volume	**20-250 µl**
Application Volume	**1-20 µl require special handling**
Application Amount	**≤ 25 µg**
Concentration (DNA application)	**Any**
Recovery Volume	**Equivalent to application vloume**
Concentration (DNA recovery)	**≤200 ng/µl**
Drying Method	**FastDryer, Vacuum, Biosafety hood**

Product Information

GenTegraDNA 0.5ml Cluster Tube Racks (Rack of 96 tubes)	
Catalog #	GTD0001; GTD0010; GTD1010-BC
Tube Volume	0.5mL
Application Volume	20-250µL
Application Volume	1-20 µl require special handling
Application Amount	≤ 25µg
Concentration (DNA application)	Any
Recovery Volume	Equivalent to application vloume
Concentration (DNA recovery)	Any
Drying Method	FastDryer, SpeedVac or Desiccator



Technical Information qPCR

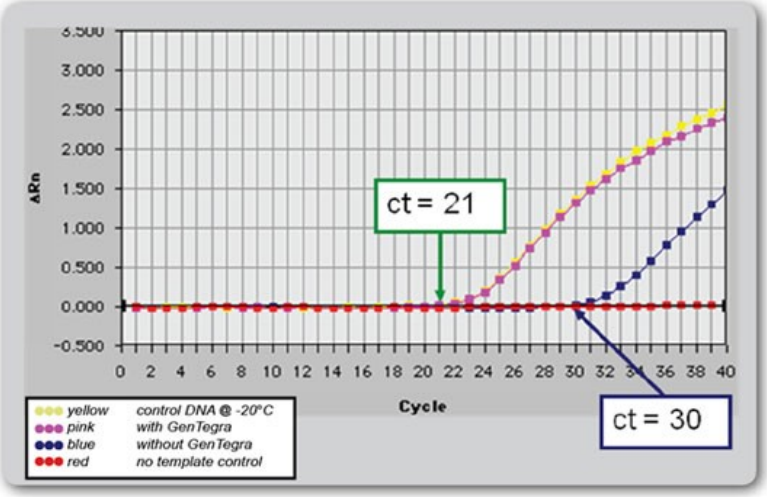


Figure 3. Successful qPCR amplification of DNA stored in GenTegraDNA Tubes. Following recovery of DNA after storage at 76°C for two weeks with GenTegraDNA , no PCR inhibition was observed even when 26% of the reaction volume was made up of DNA. The green box indicates Ct value of control DNA stored at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

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 degradatlon.[1]

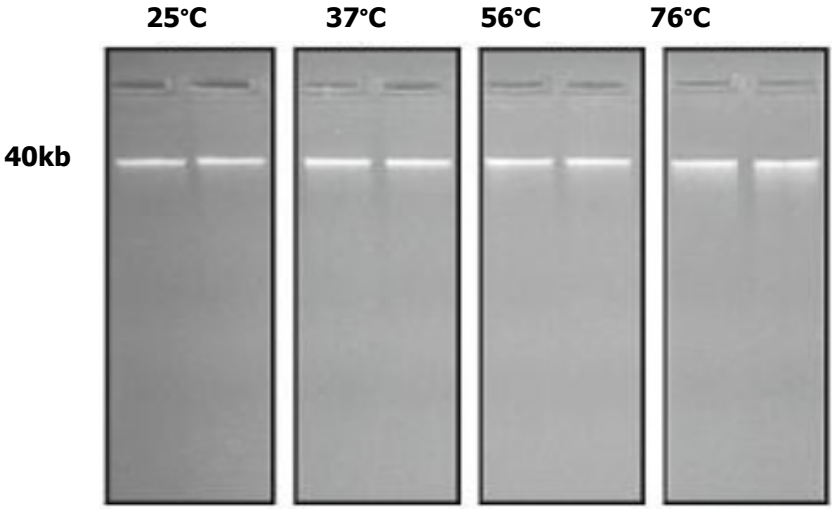
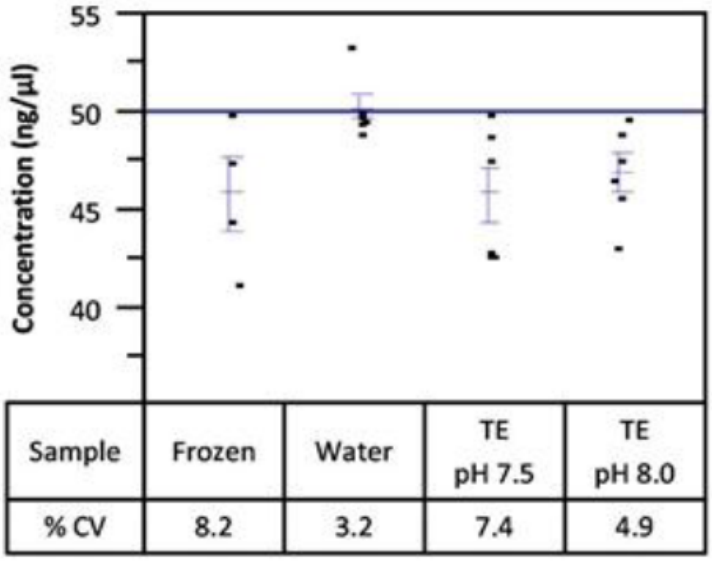


Figure 4. 250 ng/lane genomic DNA stored on GenTegra DNA for six months at ambient (25°C) and elevated temperatures.

Technical Information



Sample	Frozen	Water	TE pH 7.5	TE pH 8.0
% CV	8.2	3.2	7.4	4.9

Figure 1. DNA is quantitatively recovered from GenTegraDNA Tubes.

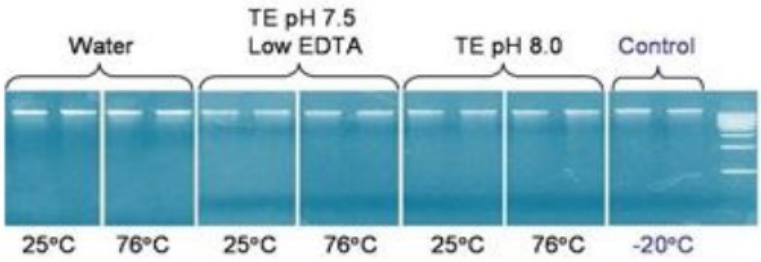


Figure 2. Quality and integrity of DNA stored in GenTegraDNA Tubes is identical to DNA stored at -20°C. DNA was stored for 120 days at room temperature (25°C) or 76°C. 120 days of storage at 76°C is equivalent to 10 years of room temperature storage.

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

		Control (-20°C)	GenTegraDNA (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 GenTegraDNA Tubes at room temperature.

Sample volumes of 20 µl or less

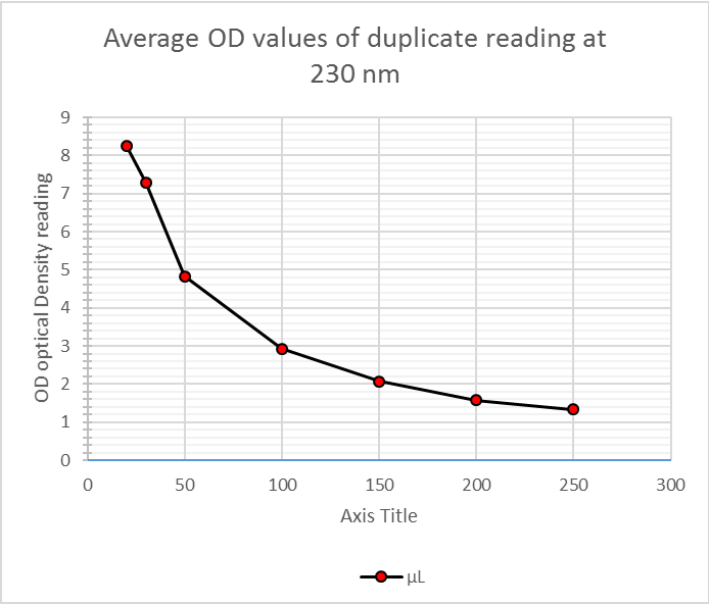
GenTegraDNA tubes, microplates and Cluster tubes all start with 21 µl of GenTegraDNA solution being added to the bottom of the tube or well followed by drying. This means the 21 µl coats the bottom and side walls of each tube/well to the height of 21 µl. If the sample volume being used is less than 20 µl it is unlikely that all the GenTegraDNA 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 GenTegraDNA. For volumes 10 µl or less this is 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 µl 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 µl low volume cut off is not because GenTegraDNA cannot protect small samples but because these small samples are difficult to process conveniently.

Correcting 260/230 ratios

The GenTegraDNA 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 GenTegraDNA solutions at differing volumes and the table below shows the numerical values.



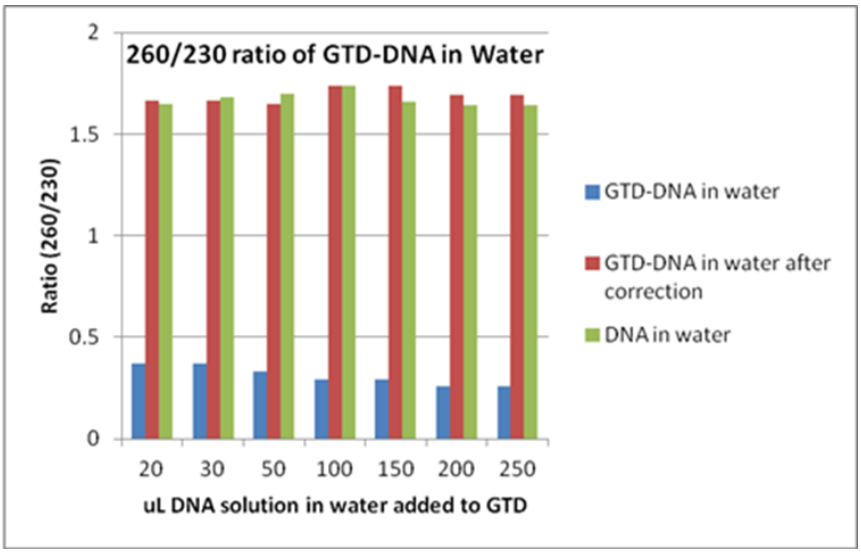
µL	OD
250	1.33
200	1.57
150	2.07
100	2.93
50	4.83
30	7.30
20	8.26

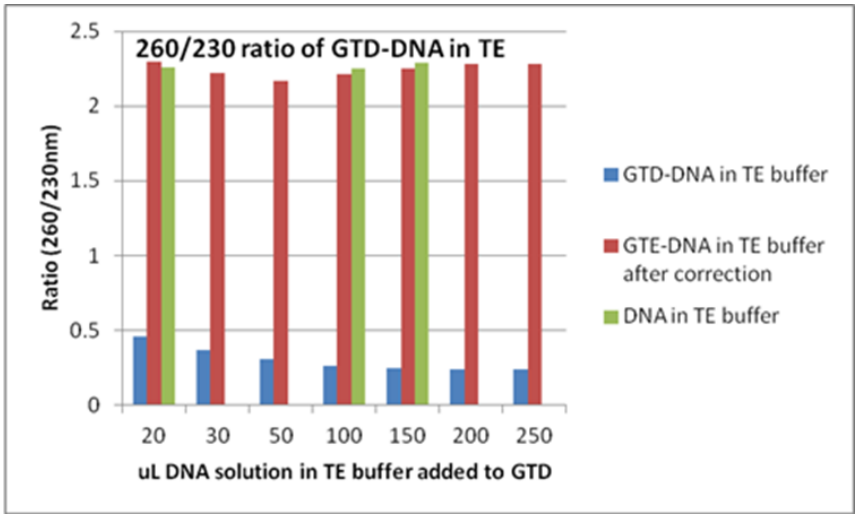
With these values it is possible to create a table of correction values that can be applied to the 260/230 ratios determined using a NanoDrop for example. The absorbance ratio is also affected if TE buffer is being used so a second column is given for the correction factor to use if the DNA & GenTegra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

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

	Correction Factor	
uL DNA added to GTD	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 GenTegraDNA 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?

GenTegraDNA is not a filter, beads or paper. GenTegraDNA is an inert chemical matrix.

The GenTegraDNA Cluster Tubes, 1.7ml Tubes, or 96- well microplates appear to be empty. Where is the GenTegraDNA and how can I detect it?

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

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

Yes, refer to Table 2 for a list of storage solutions that are compatible with GenTegraDNA 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 GenTegraDNA. 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 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 GenTegraDNA affect my DNA quantitation? Do I need to blank the spectrophotometer with the GenTegraDNA?

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

How should I store my recovered DNA?

If the recovered DNA is in GenTegraDNA 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. GenTegraDNA 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.

Toll free: 844.540.4000 • Tel: 925.461.3010 • Email: sales@gentegra.com •www.GenTegra.com

For laboratory use only.