GenTegraDNA dry BULK User Guide

GTD1000-1

Forensic Edition

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Research Use Only

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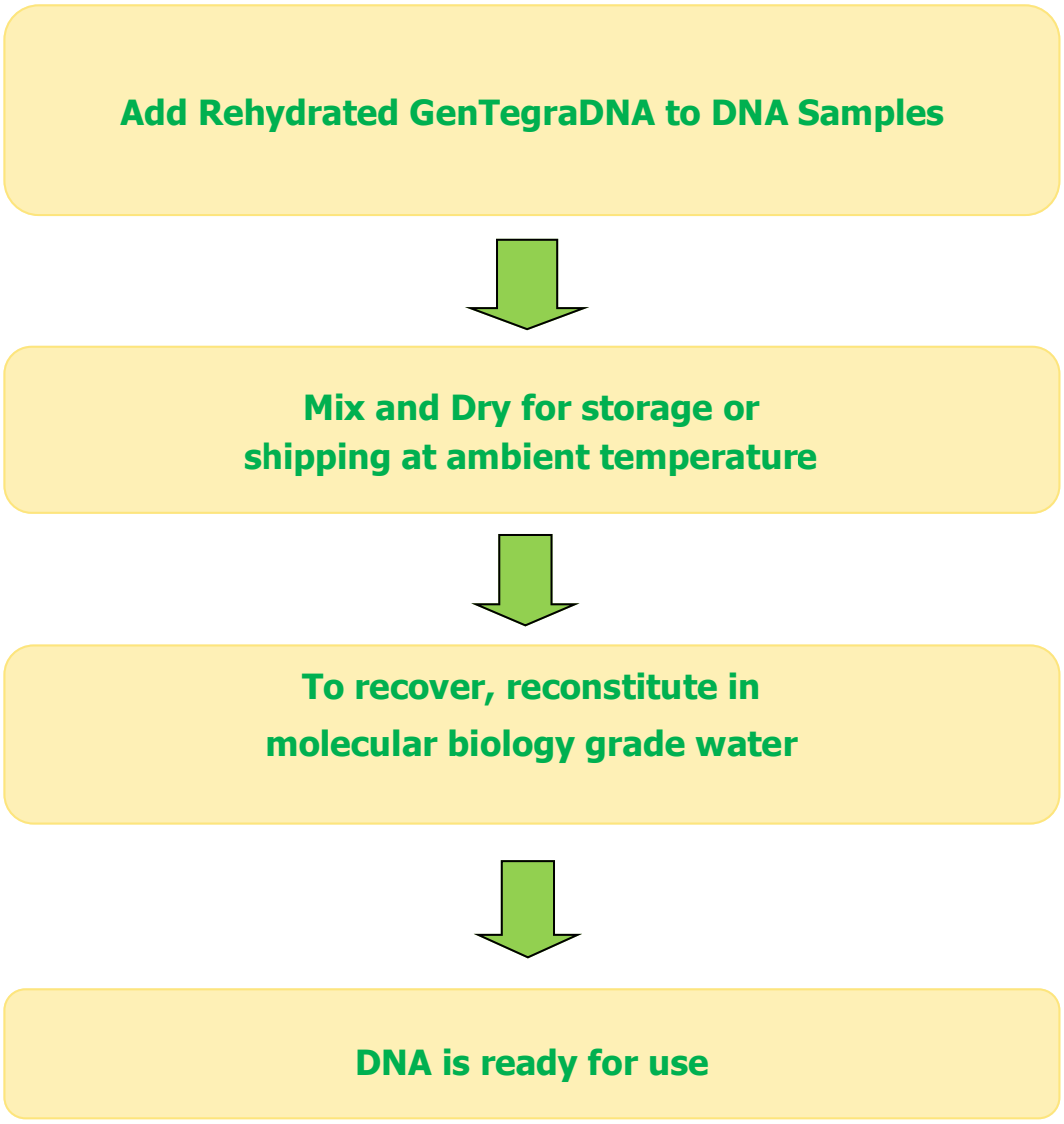
Simplified Workflow



Overview

GenTegraDNA™ is a novel technology for storage and transport of DNA in ready to use aliquots. GenTegraDNA allows storage of DNA in a water-free environment, which protects samples from hydrolysis, oxidation and microbial growth. Simply add purified DNA, dry, and store at room temperature. When needed, simply rehydrate and the DNA sample is ready for downstream analysis. GenTegraDNA 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, GenTegraDNA is available in bulk form for adding directly to liquid samples of purified DNA, followed by gentle mixing and drying.

Product Information

	GenTegraDNA Dry BULK
Catalog Number	**GTD1000-1**
Product form	**Dry material in 6 ml vial**
Sample Volume	**5-250µl**
Sample Amount	**0.01-25 µg of DNA**
DNA concentration	**Any**
Recovery Volume	**Equivalent to Sample volume**
For Forensic samples 20-250µL Typical sample size: 100-150µL	**Add 5.5mL molecular biology grade water, mix gently to dissolve**
Amount per sample	**Add 5µL to each sample**
For Forensic samples 20-175µL Typical sample size: 50-100µL	**Add 5.5mL molecular biology grade water, mix gently to dissolve**
Drying Method	**SpeedVac, Vacuum Desiccator, FastDryer™, Biosafety Hood**

Upon arrival, GenTegraDNA dry BULK is dry material in the bottom of the tube. In the dry form GenTegraDNA has a shelf life of at least three years when stored at ambient temperature (15-30°C). 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 GenTegraDNA

- 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 GenTegraDNA

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

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

GenTegraDNA Protocol – Adding to Samples

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

1. Spin the product tube briefly or tap gently on bench to drive reagents to bottom of the tube.
2. Add 5.5ml of water to the GenTegraDNA Dry BULK tube, replace the cap, and dissolve using gentle tube inversions for 15-30 minutes.
3. Add 5µl of GenTegraDNA solution to each isolated DNA sample.

   The DNA sample amounts that may be used:

   - Volume: 5-245 µl Amount: 0.01-25 µg

4. Mix thoroughly and gently to disperse the GenTegraDNA matrix and avoid foaming.
5. Quickly centrifuge to bring the matrix and sample to the tube/well bottom.
6. 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 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 stable for long term ambient storage.

Drying and Storing GenTegraDNA

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

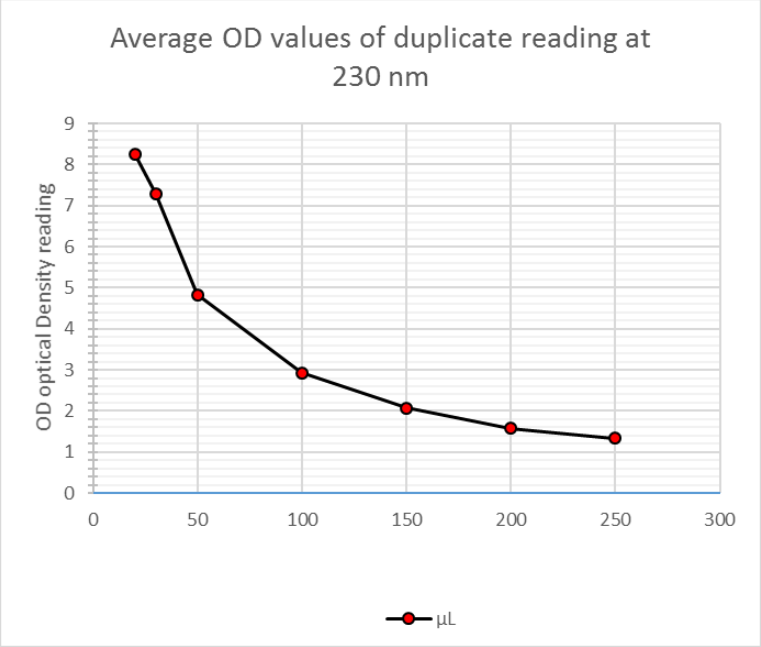
- Drying times for SpeedVac and biosafety hood vary depending on the sample volume.
  ≤50 µL - ~2hr
- When using a SpeedVac or biosafety hood, ensure that DNA is completely dry prior to storage.
- Use SpeedVac on room temperature setting (no additional heat or cooling).
- Drying times for 96-well microplates in a biosafety hood are approximately:
  ≤50 µL - 24hr;
  ≤100 µL - 48hr;
  ≤250 µL - 72hr

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, GenTegraDNA chemical matrix) is removed.
For example, a 200 µL sample is applied to a GenTegraDNA 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°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).

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 normally 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.



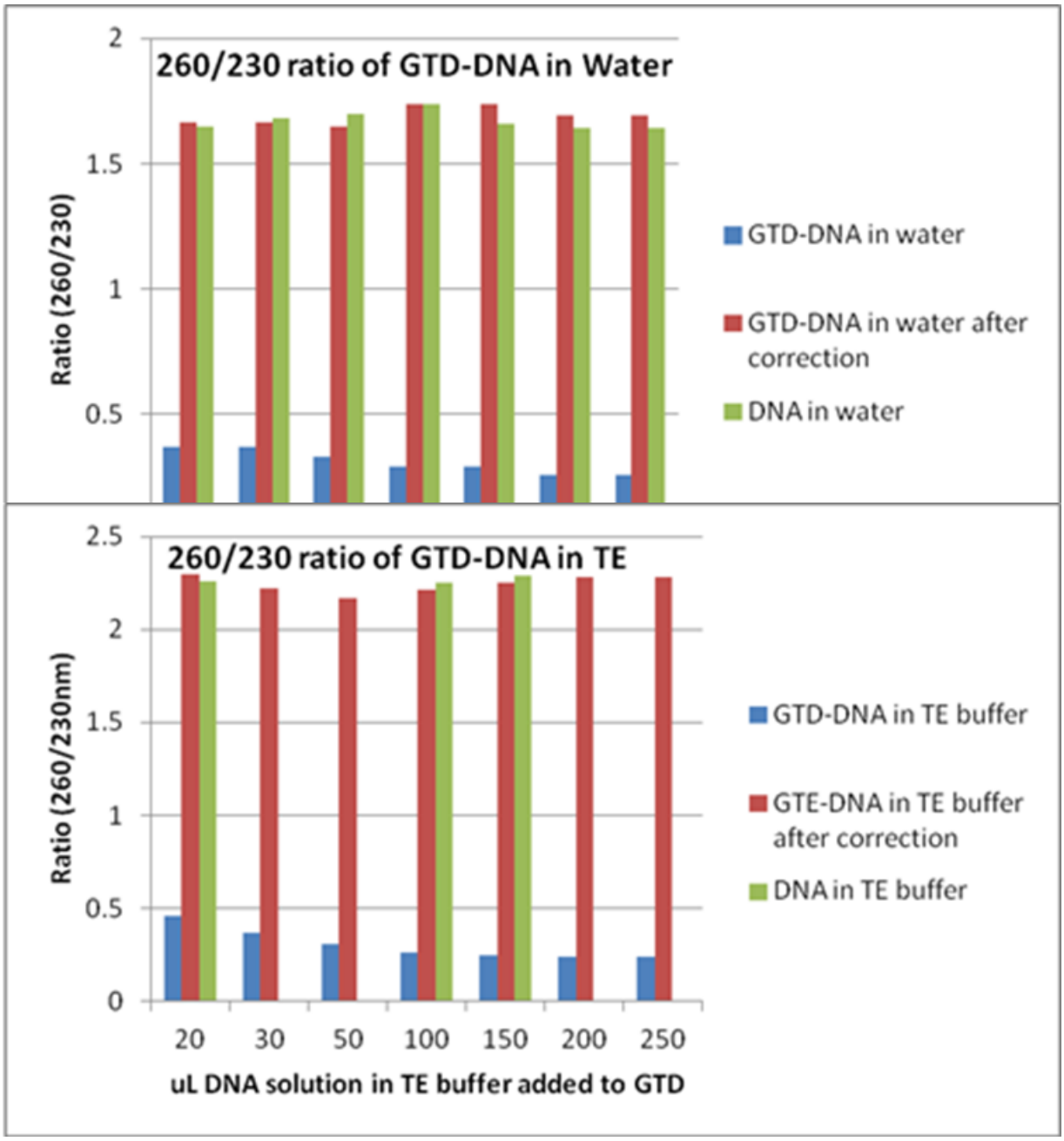
µL	OD
250	1.33
200	1.57
150	2.07
100	2.93
50	4.83
30	7.30
20	8.26

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 GenTegraDNA? Is GenTegraDNA composed of a filter, beads or paper?

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

The GenTegraDNA dry BULK tubes 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. For larger sample numbers 1,000 or more, the chemical matrix will be visible in the tube or bottle.

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 GenTegraDNA 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 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.

Frequently Asked Questions (FAQ) cont'd

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 or pretreatment 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.

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

We recommend using the 1X concentration so a larger surface area at the bottom of the tube is coated with GenTegraDNA but it is acceptable to deposit 5 µl of 5X solution in the bottom of the tube.

My DNA recovery upon rehydration seems low, what could causse 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:
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