GenTegra®-DNA User Guide

Ambient temperature storage and transport of purified DNA

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FOR RESEARCH USE ONLY

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Simplified Workflow

Apply DNA to GenTegra-DNA
Tubes/wells



Dry for storage or shipping at ambient temperature



To recover, reconstitute in Molecular-grade water for use in downstream applications



DNA is ready for use

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Summary

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, 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).

Expected Results

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

Storage and Transport

- Store and transport at ambient temperature
- DNA stored in GenTegra-DNA is designed to tolerate temperatures of -80°C to 76°C during transport.



GenTegra-DNA 0.3ml Cluster Tubes (Rack of 96 tubes)		
Catalog #	GTD1001; GTD1011; GTD1112; GTD1122	
Tube Volume	0.3mL	
Application Volume	20-250μL	
Application Amount	0.05-25μg	
Concentration (DNA application)	Any	
Recovery Volume	≤200ng/μL	
Concentration (DNA recovery)	Any	
Drying Method	FastDryer or SpeedVac	



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GenTegra-DNA 0.5mL Screw-cap microtubes		
Catalog #	GTD2025-S (Trial kit), GTD2100-S	
Tube Volume	0.5 ml	
Application Volume	20-250 μl	
Application Amount	0.05-25 μg	
Concentration (DNA application)	Any	
Recovery Volume	35-250 μl	
Concentration (DNA recovery)	≤200 ng/μl	
Drying Method	FastDryer (≤50 μl volume) SpeedVac (≤250 μl volume)	





GenTegra-DNA 1.7mL Microcentrifuge FlipTop Cap Tubes		
Catalog #	GTD2025-F (Trial Kit), GTD2100-F	
Tube Volume	1.7 ml	
Application Volume	20-250 μl	
Application Amount	0.05-25 μg	
Concentration (DNA application)	Any	
Recovery Volume	35-1000 μl	
Concentration (DNA recovery)	≤200 ng/μl	
Drying Method	SpeedVac	



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GenTegra-DNA 96-well Microplate			
Catalog #	GTD4001-P		
Application Volume	20-250 μΙ		
Application Amount	0.05-25 μg		
Concentration (DNA application)	Any		
Recovery Volume	35-250 μl		
Concentration (DNA recovery)	≤200 ng/μl		
Drying Method	FastDryer (≤50 µl volume) SpeedVac (≤250 µl volume)		
	Biosafety Hood		





Tested storage buffers compatible with GenTegra-DNA

- Water
- 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)



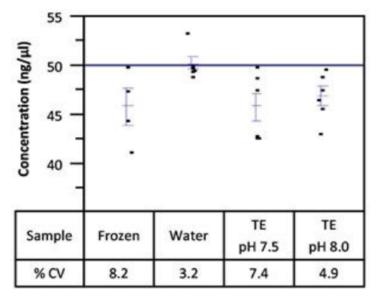


Figure 1. DNA is quantitatively recovered from GenTegra-DNA Tubes.

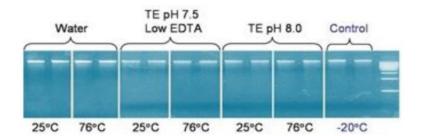


Figure 2. Quality and integrity of DNA stored in GenTegra-DNA Tubes is identical to DNA stored at -20°C. DNA was stored for 120 days at room temperature (25°C) or 76°C. 120 days of storage at 76°C is equivalent to 10 years of room temperature storage.



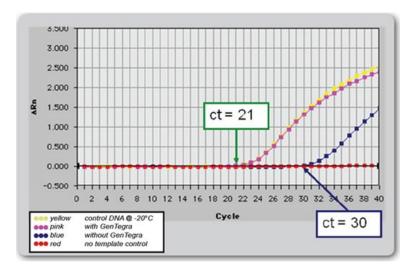


Figure 3. Successful qPCR amplification of DNA stored in GenTegra-DNA Tubes. Following recovery of DNA after storage at 76°C for two weeks with GenTegra-DNA, no PCR inhibition was observed even when 26% of the reaction volume was made up of DNA. The green box indicates Ct value of control DNA stored 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



Table 2. Successful genotyping of DNA stored in GenTegra-DNA Tubes via Illumina and Affymetrix platforms.

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



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 stoblll1y studies show DNA sample protection with no visible degradation.¹

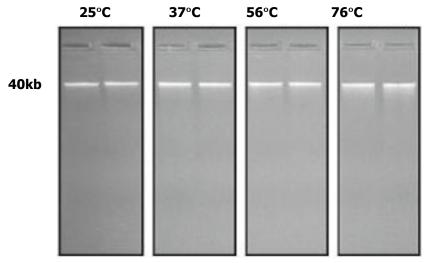


Figure 4. 250 ng/lane genomic DNA stored on GenTegra DNA for six months at ambient (25°C) and elevated temperatures.

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GenTegra-DNA Protocol

DNA Application

- 1. Apply DNA to GenTegra-DNA according to the guidelines in the table below.
- 2. Mix by pipetting up and down 6 times to solubilize the GenTegra-DNA.

GenTegra-DNA is supplied as a transparent coating at the bottom of each GenTegra-DNA Tube or well.

3. Proceed to Drying Protocol (Page 15).

Product	Catalog Number	Application Volume	Application Amount
0.3 ml Cluster Tubes	GTD1001 GTD1011 GTD1122 GTD1112	20-250 μΙ	
1.7 ml Tubes	GTD2025-F GTD2100-F		0.05-25 μg
96 Well Microplate	GTD4001-P		
0.5 ml Screw Cap Tubes	GTD2025-S GTD2100-S		



GenTegra-DNA Protocol

Drying and Storage of DNA

- 1. Dry DNA according to the methods described in the table below. Refer to Page 17 for FastDryer operation instructions.
 - Drying times for SpeedVac and biosafety hood will vary depending on application 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 biosafety hood are approximate.

When using 0.5ml screw cap tubes in a FastDryer, volume must be $\leq 50\mu 1$.

2. When drying is complete, cap or seal tubes/plates and store at room temperature ($21-25^{\circ}$ C).

Volume	FastDryer	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

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GenTegra-DNA Protocol

DNA Recovery

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

Ensure that the final concentration of DNA is $\leq 200 \text{ ng/}\mu\text{l}$.

3. Incubate at room temperature (21-25°C) for 15 minutes.

Product	Catalog Number	Recovery Volume and Concentration	Solubilization
0.3 ml Cluster Tubes	GTD1001 GTD1011 GTD1122 GTD1112	35-250 μl ≤200 ng/μl	Cap tubes and vortex for 1 minute
1.7 ml Tubes	GTD2025-F GTD2100-F		Cap tubes and vortex for 1 minute
96 Well Microplate	GTD4001-P		Pipette up and down 10 times
0.5 ml Screw Cap Tubes	GTD2025-S GTD2100-S		Cap tubes and vortex for 1 minute



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-DNA) is removed.

For example, a 200 μ l sample is applied to a GenTegra tube, dried and rehydrated. Following rehydration, 50 μ l is removed for analysis, leaving 150 μ l (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 μ l (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 μ l could undergo drying and rehydration until the volume drops below 25 μ l (25% of the original sample).



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 Cluster Tubes, 1.7ml Tubes, or 96- well microplates 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 $35\mu L$ 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 <u>application</u> (note that the maximum concentration for <u>recovery</u> is 200 ng/ μ l) When applying less than 20 μ L of DNA, add water to a final volume of \geq 20 μ l 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 35 μ l?

A minimum 35 μ l volume is required to rehydrate DNA from all surfaces of the tube or well.



Frequently Asked Questions (FAQ) cont'd

Why is there a maximum recovery concentration of 200ng/ μ L 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.

How should I store my recovered DNA?

We recommend storing recovered DNA at 4° C for short-term use and - 20° C for long-term storage. Do not store recovered DNA at room 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.



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