GenTegra®

Market leader in biomolecule protection

APPLICATION NOTE

Utilizing GenTegraDNA™ for Long-Term Room Temperature Storage of Forensic DNA Extracts

Highlights

- Easy integration into forensic workflows, including long-term storage of GenTegra protected DNA samples for further forensic casework analysis.
- An effective and superior way to concentrate DNA samples to an appropriate volume for amplification.
- No compromise or interference in downstream applications.
- Effective solution for long-term DNA sample storage that reduces DNA degradation, prevents precious sample loss, and dramatically reduces the costs, space, and added overhead for maintenance associated with failing freezer systems.

INTRODUCTION

DNA Storage Challenges Faced by Forensic Labs

Long-term storage of DNA extracts is a logistical challenge for forensic labs. Freezing is the traditional gold standard for long-term storage of extracted DNA. Long term freezer storage of extracted DNA samples comes with inherent challenges, including further loss through repeated freeze-thaw cycles, adherence to the tubes, evaporation, and degradation. In the US alone, there are hundreds of thousands of forensic DNA samples collected each year. As of October 2021, the National DNA Index System (NDIS) has collected over 20 million profiles in their database and aided over 574 thousand investigations.[1] NDIS participating labs must comply with federal regulations, including storage and preservation guidelines to prevent the loss of DNA forensic samples, set forth in the Quality Assurance Standards for Forensic DNA Testing provided by the FBI Director to access the database.[2] These samples are routinely stored and available for reanalysis for as long as the statutes of limitations apply or, as in the case of homicides or missing persons, indefinitely. Best practice for forensic casework requires that any remaining DNA extract from analysis of casework samples should be retained for possible future testing if required.

Compounding the stability and sample loss issues associated with DNA collection, extraction, and processing, freezer storage systems have their own inherent challenges. They require backup systems in case of power failures and have significant running costs for energy and maintenance. Aging freezers are subject to catastrophic freezer failures. Management and retrieval of frozen samples presents logistical challenges for the laboratory. Given all these issues, there is a need for a better storage solution that addresses these problems.

The GenTegra Solution for Long-Term DNA Storage

GenTegraDNA is a robust and reliable dry storage protection product that utilizes GenTegra's proven, patented Active Chemical Protection™ to protect DNA from hydrolysis and exposure to oxidation, and allows for higher recovery rates. GenTegraDNA provides protection even in the presence of extreme temperature conditions (-80 °C to +72 °C). Additional benefit, samples stored or shipped in GenTegraDNA do not require any specialty humidity control.

Studies have shown that GenTegraDNA protects DNA for over 20 years at ambient temperatures based on accelerated studies. For further validation, we collaborated with forensic laboratories to provide real-world examples of GenTegraDNA's protection and sample recovery for downstream analyses.

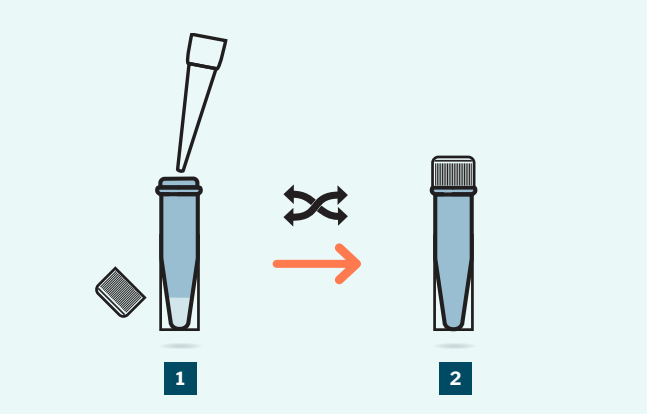
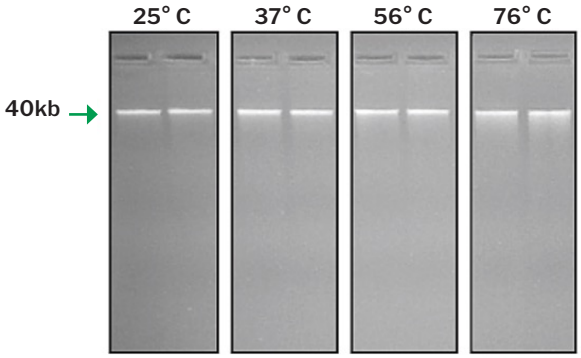


Figure 1: GenTegraDNA protects DNA in 2 simple steps. **1.** Combine DNA extractions with GenTegraDNA solution in a screw cap tube and gently mix. **2.** Dry the combined DNA with GenTegraDNA sample solution. (Recommended drying methods are provided in the Product Specifications Table at the end of this Application Note with additional instructions provided in the GenTegraDNA User Guide.) Following long-term storage, DNA can be easily recovered and ready for use in downstream applications without the need for additional purifications.

GenTegraDNA Delivers Long Term Protection and Stability

To determine GenTegraDNA's ability to stabilize DNA for long-term storage, extracted DNA samples were protected with GenTegraDNA and then dried down for testing. Using the Arrhenius model for accelerated stability testing, samples were stored at 25 °C (room temperature), 37 °C, 56 °C, and 76 °C for 6 months. Following the 6-month storage, 250 ng of DNA sample was analyzed by gel electrophoresis, where no visible degradation was observed (Figure 2). Storing the DNA at 76 °C for 6 months is the equivalent to 20 years of storage at ambient room temperature.[3,4]



250 ng/lane genomic DNA stored on GenTegraDNA for six months of ambient (25°C) and elevated temperatures.

Figure 2: DNA samples stored on GenTegraDNA show no degradation after the equivalent of 20 years storage at ambient temperature. Accelerated stability studies show DNA sample protection with no visible degradation.

For Forensic DNA:

Stability and Recovery Analysis of DNA Extracts Protected with GenTegraDNA

To validate GenTegraDNA for subsequent storage of forensic DNA samples, an independent forensic lab performed a series of experiments examining quality assurance parameters based on guidelines for use in forensics cases.

These studies were conducted over a period of 76 days and included:

- A dilution series of 1:10,000 of a non-degraded reference sample extract with aliquots stored under the following conditions:

  a) Unprotected DNA samples in a freezer;

  b) GenTegraDNA protected samples at room temperature,

  c) GenTegraDNA protected samples in a 70 °C incubator for accelerated stability assessment
- A set of degraded extracts with aliquots stored under the following conditions:

  a) Unprotected DNA samples in a freezer;

  b) GenTegraDNA protected samples at room temperature,

  c) GenTegraDNA protected samples in a 70 °C incubator for accelerated stability assessment
- A range of extracts starting volumes (5 µL to 40 µL) stored at room temperature

All studies above were tested for amplification with GlobalFiler™ (ThermoFisher Scientific) and/or PowerPlex® Y23 (Promega) to determine data quality standards were met with the respective samples.

Accelerated stability testing of non-degraded reference sample extract and a set of degraded extracts

As shown in Table 1, samples protected with GenTegraDNA showed a higher to equivalent percent of recovery at both room temperature and 70 °C during accelerated stability testing. In addition, samples protected with GenTegraDNA were observed to have little to no additional degradation as indicated by the Degradation Index (DI). The DI is a way to evaluate degradation in forensic samples and is the ratio of the number of small DNA fragments to larger DNA fragments in a sample. Dilutions 1:1,000 and 1:10,000 (grey) are expected to be subject to stochastic effects with Quantifiler Trio.

	Initial T-S (theory)	No GenTegraDNA -20 °C freezer		GenTegraDNA room temperature		GenTegraDNA 70 °C	
Sample	**Sample input**	**% Recovered**	**DI**	**% Recovered**	**DI**	**% Recovered**	**DI**
Undiluted (neat)	1.8 ng	86%	1.2	128%	1.3	113%	1.2
1:10	0.18 ng	44%	1.3	49%	1.4	78%	1.7
1:100	0.018 ng	54%	1.4	67%	1.9	57%	2.4
1:1,000	0.0018 ng	67%	1.3	100%	4.5	11%	0.7
1:10,000	0.00018 ng	111%	2	222%	———	0%	———

Table 1: Quality and recovery assessment of dilution series of a DNA reference sample for forensic analysis. In samples protected with GenTegraDNA, the percent recovered at both room temperature, and 70 °C testing showed a higher to equivalent percent of recovery. In addition, samples showed little to no additional degradation. The DI value of <1 suggests intact DNA with no degradation or inhibition. DI values between 1-10 indicate that DNA is slightly to moderately degraded but would not likely result in PCR inhibition, and DI values > 10 are indicative of significant degradation. Values in grey are expected to be subject to stochastic effects with Quantifiler Trio (the method used in this study for quantifying DNA samples).

Based on quantification results from the titration series, the undiluted and 1:10 dilution points from each set of conditions were amplified with both GlobalFiler and PowerPlex Y23, and 1:100 dilutions were only amplified with PowerPlex Y23. Complete profiles were obtained for ≥ 0.18 ng samples. Partial profiles were obtained for 0.018 ng (data not shown).

An additional set of degraded extracts (as an example of potential forensic sample types) were retained under the same set of storage conditions with the following results in Table 2.

	Before storage		No GenTegraDNA -20 °C freezer		GenTegraDNA room temperature		GenTegraDNA 70 °C	
Sample	**Sample input**	**Initial DI**	**% Recovered**	**DI**	**% Recovered**	**DI**	**% Recovered**	**DI**
1G	3.003	2.0	70%	1.5	75%	1.7	77%	2.1
1I	2.901	3.2	81%	3.5	72%	3.5	69%	3.8
1J	1.180	1.4	89%	1.9	66%	1.6	80%	1.9
1K	0.537	1.2	30%	2.1	45%	2.1	62%	2.4
1H	0.252	28.0	46%	21.5	50%	39.1	57%	30.6

Table 2: Quality and recovery assessment of DNA degraded samples for forensic analysis. In samples protected with GenTegraDNA, the percent recovered at room temperature and 70 °C for accelerated stability testing showed a comparable percent of recovery and no significant degradation in samples with a starting input > 1 ng/µL. The samples with starting inputs < 1 ng/µL, percent recovery showed a notable increase with no significant increase in sample degradation.

These results indicated a trend that concentrations above 0.1 ng/µL with GenTegraDNA appears to support greater recovery following storage. Storing DNA frozen (e.g., gold standard) or with GenTegraDNA, at room temperature and 70 °C, during the tested period (76 days) does not lead to significant degradation. Overall, these findings suggest comparable results for storage by freezer or room temperature with GenTegraDNA in terms of DNA recovery and quality.

Contamination analysis

Reagent blanks for each of the above studies were quantified with no detected DNA. Reagent blanks were amplified with either GlobalFiler or PowerPlex Y23. GlobalFiler blanks yielded profiles with no detected DNA. PowerPlex Y23 blanks also produced profiles with no detectable DNA but consistently showed at least one “haystack”-shaped artifact between 75 and 76 base pairs.

Long-term Storage and DNA Integrity for Downstream Applications

All forensic cases require DNA samples to remain intact for future testing. These studies were conducted to confirm GenTegraDNA prevents degradation over long-term storage at ambient temperatures and samples remain intact for downstream applications and analysis.

Ongoing Ambient Temperature Experiments Show Retention of Sample Quality Through 4.5 Years

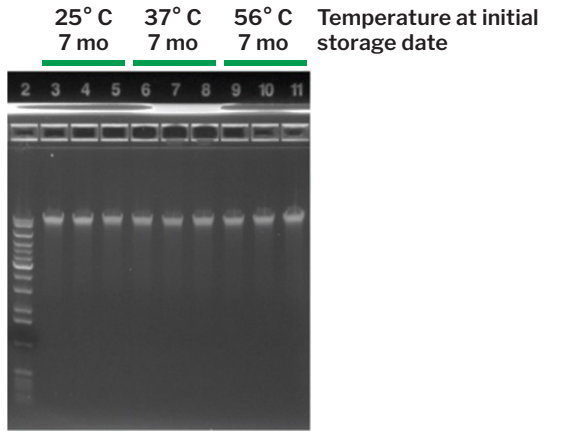
Following the initial six-month incubation period at 25 °C, 37 °C, and 56 °C for accelerated stability testing with GenTegraDNA, mitochondrial DNA samples were moved to storage at room temperature (25 °C) for four years, thus replicating normal storage conditions. These samples showed no degradation and performed identically in downstream applications (Figure 3).

qPCR Amplification is Not Inhibited by GenTegraDNA

To confirm that GenTegraDNA does not interfere with fluorescence-based reporter dyes during qPCR amplification, samples protected with GenTegraDNA were compared to unprotected DNA samples. 20 µL of 10 ng/µL DNA was added to 6 control tubes without GenTegraDNA and 6 tubes with GenTegraDNA. All DNA samples were analyzed in triplicate with TaqMan® PCR (ThermoFisher) using GAPDH primer and probe (FAM-BHQ) set. No differences were observed between GenTegraDNA treated samples and untreated samples demonstrating that GenTegraDNA does not inhibit real-time TaqMan PCR with FAM as a reporter dye (Figure 4).

qPCR assay validated long-term protection of DNA integrity with GenTegraDNA

Following degradation analysis, the 4.5-year GenTegraDNA-protected extract samples were amplified by qPCR with long-range PCR primers. In this study, the DNA extracts were observed to be intact to at least 2.5 kb with normal C_t values (19–20 cycles) for all samples (Figure5).



100 ng/lane genomic DNA stored on GenTegraDNA for four years following incubation at three different temperatures.

Figure 3: Agarose gel assessment of genomic DNA quality. Following storage, the samples were rehydrated and visualized using agarose gel electrophoresis. A (collapsed) DNA band with an apparent molecular weight of 40 kb indicates that the average duplex DNA strand length for such samples is in excess of approximately 40 kb.

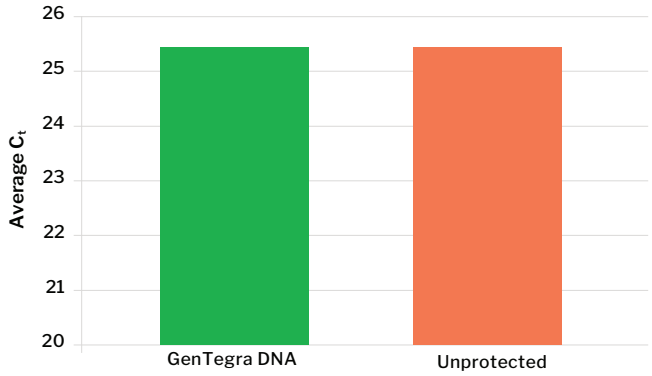


Figure 4: GenTegraDNA does not inhibit qPCR amplification. C_t values showed normal amplification with GenTegraDNA-protected samples compared to unprotected samples.

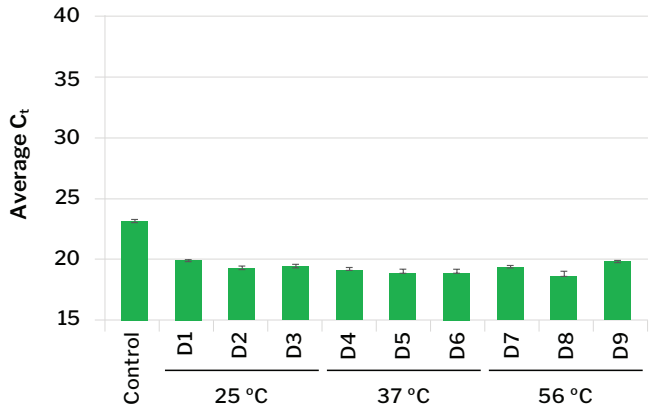


Figure 5: GenTegraDNA protects integrity of DNA samples for qPCR. C_t values showed normal amplification with long-range PCR primers on GenTegraDNA-protected extract samples stored for 4.5 years.

Using GenTegraDNA for Concentrating Forensic DNA Samples to Improve Recovery

A reliable DNA concentration method allows for combining extracts and using the most DNA for testing while leaving a portion for future testing. Traditional filters used for sample concentration can result in sample loss and add the potential for sample switching due to tube transfers. Therefore, a study was conducted to validate an alternative drying process for concentrating DNA samples.

In this study, two dry-down methods were tested for concentration and recovery of DNA. For both methods, initial total DNA input was quantified for all samples.

- **Method 1:** No DNA stabilization protection in a 60 °C incubator
  - 12 DNA samples with 5 high quantity **(Figure 5A, orange)** and 7 relatively low quantity **(Figure 5B, orange)** inputs of less than 1 ng
  - For contamination testing, 6 samples containing only molecular biology grade water were placed between 2 rows of DNA containing samples
  - All samples were dried down without DNA stabilization protection in a 60 °C incubator for 1 hr 40 min to volumes between 7 µL and 11 µL
  - Sample volumes were brought back up to 15 µL with molecular biology grade water
- **Method 2:** GenTegraDNA protection with overnight dry-down
  - 7 DNA samples with 3 high quantity **(Figure 5A, green)** and 4 relatively low quantity **(Figure 5B, green)** total input
  - DNA samples were added to GenTegraDNA protection tubes and subsequently completely dried down overnight in a 60 °C incubator
  - Following dry down, the GenTegraDNA-protected samples were subsequently reconstituted with 15 µL of molecular biology grade water

Following concentration, all DNA samples were quantified, and the degradation index was determined with Quantifiler Trio. The total amount of DNA after dry-down was determined by multiplying the concentration by 15 µL.

For both dry-down processes, sample quantity and degradation were analyzed. **For the first method**, all high quantity samples showed some DNA loss following dry-down compared to their initial input (Figure 5A, and Table 3) and 6 of the 7 of the low quantity samples showed significantly less total DNA following dry-down and reconstitution. Note, two samples showed a significant increase in sample degradation and were determined to be unacceptable for use (Table 3, *starred examples). For confirmation of no cross-contamination during drying, the blanks were quantified and showed that no DNA was present (data not shown). Thus, confirming there was no cross-contamination during the drying process.

For the GenTegraDNA protected dry-down method, 2 of the 3 higher level samples showed higher total DNA quantities (Figure 6A). The degradation index showed a modest increase in 1 of the 3 samples and no significant change in the other 2 samples (Table 3). In contrast, lower-level samples had an average detection of 74% (Figure 6B) and the DI showed no significant difference with 2 samples and a modest increase with the remaining 2 samples (Table 3).

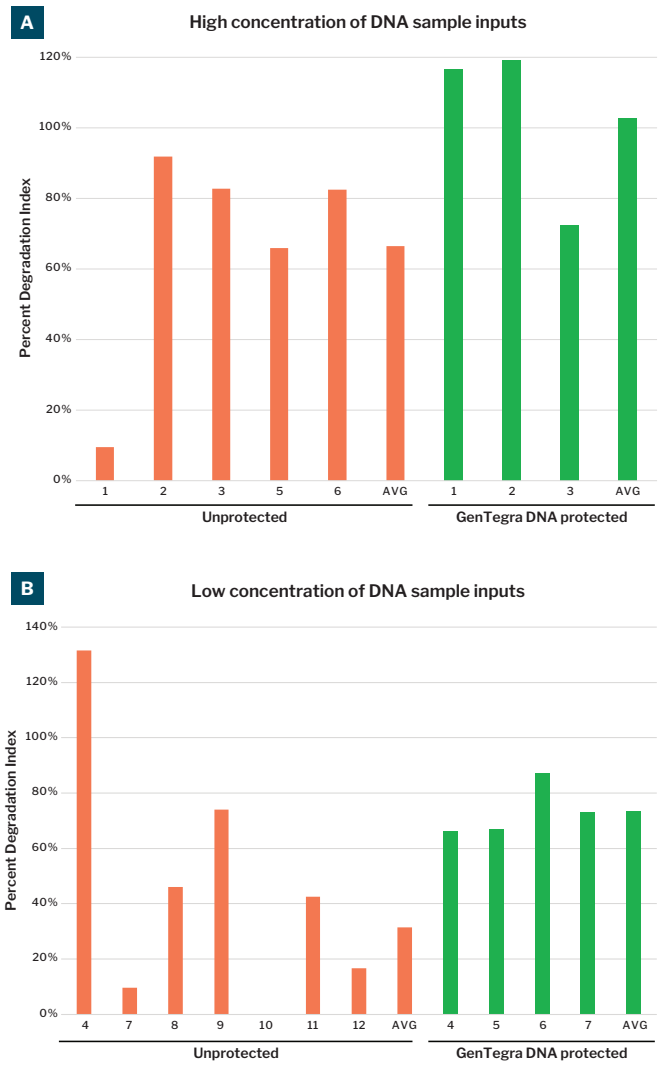


Figure 5: Samples with either high (A) or low (B) starting inputs were analyzed for percent recovery following GenTegra-protected and unprotected dry-down concentration methods. A) In method 1, five samples (orange) were unprotected during the dry-down process. In method 2, three samples (green) were protected with GenTegraDNA for the dry-down concentration process. The GenTegraDNA samples had an overall higher percent of recovery compared to unprotected DNA samples following reconstitution. **B)** In method 1, seven samples (orange) were unprotected during dry-down and compared to method 2 with four samples (green) protected with GenTegraDNA for the dry-down concentration process. Overall, the GenTegraDNA samples had a higher percentage of recovery compared to unprotected DNA samples following reconstitution.

Following this study, the investigators concluded that **"GenTegra [DNA] dry-down [method is] an effective way to concentrate samples to an appropriate volume for amplification. They are superior to dry-down techniques without a stabilizing agent and exhibit minimal DNA loss. They also allow for dry-down post examination for effective DNA extract storage."**

A. Samples with high concentration and quantity of DNA

		DNA quantity (ng)		% Recovery	DI		DI Ratio
	Samples	Total Before	Total After	After/Before	Before	After	After/Before
Unprotected	1	6.72	0.64	9.52%	1.40	1.23	0.88
	2	135.49	124.37	91.80%	0.99	1.01	1.02
	3	613.54	507.46	82.71%	1.08	0.95	0.88
	5	84.29	55.55	65.91%	1.26	0.99	0.79
	6	85.14	70.17	82.43%	1.38	1.41	1.02
GenTegra DNA	1	61.69	71.88	116.51%	0.80	1.07	1.33
	2	338.12	403.47	119.33%	1.05	1.01	0.96
	3	39.05	28.26	72.38%	1.16	1.21	1.04

B. Samples with low concentration and quantity of DNA

		DNA quantity (ng)		% Recovery	DI	
	Samples	Total Before	Total After	After/Before	Before	After
Unprotected	4*	0.01	0.01	131.56%	0.75	8.98
	7	0.57	0.01	9.56%	1.86	1.65
	8	0.27	0.13	45.98%	0.95	1.15
	9*	0.92	0.68	73.97%	7.59	13.96
	10	0.55	0.00	0.00%	14.03	————
	11	0.12	0.05	42.49%	2.08	2.12
	12	0.02	0.00	16.66%	————	2.46
GenTegra DNA	4	0.32	0.21	66.45%	0.97	1.85
	5	0.25	0.17	67.18%	1.47	1.53
	6	0.13	0.12	87.21%	1.17	2.72
	7	0.15	0.11	73.20%	1.50	1.59

Table 3: Samples were analyzed for percent recovery and degradation following dry-down concentration methods. **A)** A high concentration of DNA was used to replicate samples with an abundant amount of DNA. **B)** In comparison, DNA with a low concentration was used to replicate samples with limited sample input. GenTegraDNA protected samples showed no significant degradation. *Samples were deemed unacceptable for forensic use.

CONCLUSIONS

DNA forensics is a vital tool for crime scene analysis. While the technology and methods for the analysis of the forensic DNA samples have vastly improved, long-term storage solutions and methods for concentrating trace DNA samples have remained limited. With the current challenges and costs associated with long-term storage in freezer systems, it is evident that a cost-effective, reliable alternative to these cold storage systems is critical.

This application note shows that GenTegraDNA is a robust, reliable solution for both long-term storage and concentration of trace DNA samples. In both forensics case collaboration studies, GenTegraDNA was validated and approved for vital forensic analysis workflows, including long-term storage of GenTegra protected DNA samples and as a tool for concentrating trace samples of DNA for further forensic casework analysis. In addition, GenTegraDNA does not compromise data generated in downstream applications commonly used in forensics. GenTegraDNA protection is an effective solution for long-term storage of DNA samples that will minimize DNA degradation and prevent precious sample loss and dramatically reduce costs, space, and added overhead for maintenance associated with failing freezer systems.

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GenTegraDNA™

Product Specification	Description
Total DNA application amount	0.00 µg – ≤20 µg
Sample application volume	20-250 µL (special handling required for volumes < 20 µL)
Recovery volume	Equals application volume (20 – 250 µL of molecular biology water)
Stability for transport	Tolerance for extreme temperatures and extreme temperature shifts (-80 °C to 76 °C) Exceeds Military specifications (-60 °C to 71°C) Exceeds Federal Express® specifications (-51 °C to 60 °C)
Shelf life	3 years (prior to use)
Drying method and time	FastDryer™: Overnight SpeedVac®: 2 – 4 hours, depending on volume/type of SpeedVac Under Biosafety Hood: 14 hours
Recovery	>95%

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