

Clonestable[®] Handbook

Preserve and store bacterial DNA samples at room temperature

Protocols for Sample Protection, Sample Recovery and
Downstream Applications



Contents

Product Formats	3
Introduction	4
Principles and Procedures	4
Storage	5
Applications	6
Important Notes	7
Protocol: Storage of DNA in bacterial suspension.....	8
Protocol: Recovery of bacterial DNA.....	12
Protocol: Downstream applications	13
Troubleshooting Guide.....	14
Quality Control	18
Product Use Limitations	18
Warranty and Satisfaction Guarantee	18
Technical Assistance	21
Literature Citation	21
Safety Information.....	21

Product Formats

CloneStable®

Format*	Catalog Number	Description	Usage
96-well plate	90121-007	96-well standard footprint	Low & High Throughput labs
Individual tubes	93121-007	1.7 ml tubes	Individual samples

*All formats are supplied with barcodes.

*All formats have identical capacity, 5-20µl of *E. coli* cell culture.

Accessories for Long-term Sample Storage

Sample Pouches/Desiccant Packs, Catalog number **14001-087**. Sold in sets of 10.

Desiccant Chambers:

Dry Storage Cabinet, Catalog number: **95905-058**

Maximum capacity of (84) 96-well plates or (15) tube racks; LxWxH : 8.25x10.25x11.13,

Including (2) sorbent systems, (1) Mini hygro-thermometer

Dry Storage Cabinet, Medium with racks, Catalog number: **95904-158**.

Medium cabinet and (5) cabinet racks

Including (4) sorbent systems, (1) Mini hygro-thermometer

Dry Storage Cabinet, Large with racks, Catalog number: **95905-158**.

Large cabinet and (10) adjustable cabinet racks

Including (8) sorbent systems, (2) Mini hygro-thermometer

Cabinet Rack, Catalog number: **95904-058**.

Rack for dry storage cabinets.

Hold up to (10) multi-well plates. Dimensions: 4"x5"x8"

Introduction

Biomatrix provides innovative technology for biological sample storage at room temperature. Room temperature storage and DNA sample stability provides unique cost savings and facilitates ease of sample experimentation. CloneStable technology was designed by combining extremophile biology and synthetic chemistry to create a novel dissolvable matrix optimally formulated for long-term ambient temperature storage and shipping of bacterial DNA, both genomic and plasmid.

Recovery of bacterial DNA from CloneStable is as simple as “just adding water.” Bacterial cells do not survive more than four weeks after dry storage in CloneStable. Bacterial genomic and plasmid DNA are preserved and protected from degradation. Rehydrated samples are ready for immediate use; for most applications, no further purification is needed. Downstream applications using recovered samples include transformation, and PCR analysis. Samples stored dry in CloneStable are safe and convenient for shipping at ambient temperatures, particularly for extended transit times.

Principles and Procedures

CloneStable is a mixture of dissolvable compounds that stabilizes DNA at room temperature. The matrix formulation is based upon the natural principles of anhydrobiosis and synthetic chemistry. Meaning “life without water,” anhydrobiosis is a biological mechanism employed by some multi-cellular organisms that enables their survival in a dry state of biological stasis for periods up to 120 years (Crowe et al., *Ann. Rev. Physiol.* 1998. 60:73); during these extended dry periods, proteins, nucleic acids, membranes, and cellular systems are protected and can be revived by rehydration.

Based on the unique thermo-stable properties of Biomatrix’s proprietary formulation, CloneStable forms a protective barrier around DNA as it dries, effectively “shrink-wrapping” the sample in a protective coating. Drying can occur at ambient temperatures, with no need for special equipment. Maintained dry at ambient temperatures, the protected DNA can be safely stored for extended time periods. Currently, Biomatrix has more than two years of real-time data on stable storage of bacterial DNA in CloneStable.

Accelerated aging studies indicate that DNA can be safely stored for greater than 11 years at room temperature. Bacterial DNA samples can be recovered from CloneStable through simple rehydration and are ready for immediate use, without the need for further purification.

Storage

CloneStable is a novel formulation for safe, stable and convenient storage of bacterial DNA at room temperature. CloneStable products and kits should be stored dry in their original unopened packaging at ambient laboratory temperatures (*i.e.* room temperature at 20-25°C or 68-77°F) and at a relative humidity of less than 40%. Products and kits are supplied with moisture-barrier foil bags along with a desiccant packet. The sample pouch allows storage even in uncontrolled environments with elevated relative humidity above 40%. The packaging provided with the product is designed for proper storage using the re-sealable closure; open the packages just before use. Store any unused products in their original packaging at room temperature in a clean, dry environment.

Note: Relative humidity above 40% for extended periods of time will reduce product performance and sample protection in CloneStable. Climate-controlled laboratory environments and buildings are normally maintained at 40-50% relative humidity levels. If required, desiccating chambers are available to maintain the storage environment at ≤40% humidity. Please visit www.biomatrica.com for ordering information on desiccating chambers or extra sample pouch/desiccant packs.

Applications

Sample Type:

CloneStable has been optimized for the storage and recovery of total plasmid and genomic DNA harbored in *E. coli* bacterial strains routinely used in life science research (e.g. DH10B, DH5 α , Stbl2, etc.). This product was not tested for use with other bacterial species.

Assay Type

DNA recovered from *E. coli* after dry storage in CloneStable is ready for immediate use in downstream applications (e.g., PCR, transformation, invitro-transcription). Recovered, transformed plasmid DNA grown in fresh liquid culture can be used for cloning, sequencing, restriction analysis etc.

Shipping:

CloneStable provides the ideal format for transport and shipping of samples at ambient temperatures. Individual tubes or plates can be shipped conveniently in envelopes without the need for cold packs, dry ice, or styrofoam[®] packing, thus greatly reducing shipping costs. Fluctuating temperatures or delays during transport do not affect bacterial samples protected in CloneStable.

Important Notes

Please take a few moments to read this handbook carefully before beginning the sample preparation for dry storage of bacterial DNA at room temperature.

Sample Application and Drying

Bacterial suspensions (*e.g.* overnight cultures) applied to wells or tubes containing CloneStable must be dried completely for optimal room temperature storage. Sample volumes of up to 20 μ l can be applied directly into a CloneStable tube or well and dried at ambient temperatures on the lab bench, in a laminar flow hood (recommended) or in a SpeedVac[®] for faster drying (see pg. 9 for details).

Recovery of Bacterial DNA

Just add water or your choice of buffer to recover DNA stored dry in CloneStable. Samples are ready for immediate use in most downstream applications. It is not necessary to further purify rehydrated samples. Aqueous solutions such as TE buffer (10 mM Tris, 1 mM EDTA), PCR reaction buffers and transfection media are also compatible with the recovery of samples from CloneStable.

Cell Viability

Some bacterial cells can remain viable for up to one month after initial dry down in CloneStable. We do not recommend using samples stored for less than a month in CloneStable for transformation into a new strain of bacteria due to the risk of carry-over of the old strain. If it is preferred to store non-viable bacteria, heat the bacterial suspension at 80°C for 5 minutes to lyse the cells.

Protocol: Storage of DNA in bacterial suspension

Aliquots of *E. coli* growth cultures or suspensions can be applied directly into CloneStable for storage of total bacterial DNA. The bacteria should be added directly into CloneStable as a liquid suspension to facilitate contact between the sample and the protective matrix.

The growth medium (*i.e.* LB medium) and selective antibiotics (*e.g.* ampicillin, kanamycin) do not interfere with the storage capacity of CloneStable so there is no need to purify or wash the bacterial suspension.

Note: Glycerol stocks should not be added directly to CloneStable. See page 10 for a detailed protocol on transfer of glycerol stocks for storage in CloneStable.

Procedure

- 1. Calculate the amount of *E. coli* or total DNA to be stored in each well or tube containing CloneStable.**

Each well or tube of CloneStable can accommodate *E. coli* bacterial growth cultures in log or stationary phase with cell densities as high as $OD_{600} = 4.0$. We recommend spotting 20 μ l of a culture with $OD_{600} = 2.0$ ($\sim 2 \times 10^9$ cells/ml) onto CloneStable. This gives a final cell density of $\sim 4 \times 10^7$ cells per well.

Heat Lysis (optional): Bacteria are viable in CloneStable in a dried state for up to a month. If the sample will be used for transformation into a new strain within the first month of storage heat the bacterial suspension at 80°C for 5 minutes prior to application to CloneStable to completely lyse the cells.

- 2. Remove the plastic seal (plate format) or cap (tube format) and gently apply the sample into the center of the vessel containing CloneStable. Allow the matrix to be hydrated by the aqueous sample for 5-10 minutes.**

CloneStable is supplied as a coating on the bottom of each well or tube. The aqueous sample will rehydrate the CloneStable within minutes and form a protective barrier around the bacterial DNA as it dries.

The final volume of the sample to be applied to each well should be $\leq 20 \mu$ l.

- 3. Mix the sample thoroughly with gentle pipetting (avoid forming air bubbles).**

Alternatively, mixing can be done using a high speed shaker. Tightly secure the plate onto the shaker. Mix at 800 rpm for 30 seconds.

4. Dry the uncovered sample completely at room temperature.

We recommend using a laminar flow hood or drying with a vacuum concentrator (e.g. SpeedVac®). Recommended drying times are provided in Tables 1 and 2.

Note: Drying should occur at ambient laboratory temperatures (20°C–25°C) with relative humidity below 40%. We recommend drying under a vacuum if conditions exceed these parameters.

Table 1. Drying times in laminar flow hood

Sample Volume	Drying Times
1-5 µl	2 hours
5-10 µl	6 hours
10-20 µl	10-12 hours

Table 2. Drying times with a SpeedVac®

Sample Volume	Drying Times
10-20 µl	0.5 hours

*CloneStable is designed for optimal protection and recovery for sample volumes of ≤ 20 µl.

5. Cover samples after drying and store at room temperature.

After complete sample drying, plates should be re-sealed with aluminum foil seals (provided in kit). The aluminum seal will provide an additional moisture barrier to protect the sample. Tubes can be closed using the snap-cap. Place the plates or tubes in a moisture-barrier bag along with a silica gel desiccant packet.

Samples properly sealed can be stored at ambient temperature with relative humidity below 40%. If these environmental conditions cannot be maintained store samples in a desiccating chamber.

Protocol: Storage of DNA from glycerol stocks

Glycerol stocks containing *E. coli* strains can be transferred from cold storage into CloneStable for short term room temperature storage. The glycerol must be removed prior to storing the bacterial cells in CloneStable. Residual glycerol will interfere with complete drying of CloneStable.

Due to interference from the residual glycerol stocks, better stability is obtained by growing glycerol stock cell in an overnight culture containing appropriate media and selective antibiotic. After testing the culture for appropriate plasmid content it can then be spotted into CloneStable for long term storage.

Procedure

- 1. Thaw glycerol stock and separate cells from the glycerol solution by centrifugation at 6,000 x g for 2 min. Remove the supernatant.**

A cell pellet is formed with clear solution above it. If the solution appears cloudy, spin the sample for a longer period of time.

Stocks with high concentrations of glycerol (>50%) must be diluted with water so that the glycerol content is <50% before pelleting the cells. Cells will not pellet if the glycerol solution is too viscous.

- 2. Resuspend pellet in desired volume of water.**

We recommend resuspending the pellet to an $OD_{600} = 2.0$ ($\sim 2 \times 10^9$ cells /ml) and spotting a 20 μ l volume onto CloneStable. This gives a final cell density of $\sim 4 \times 10^7$ cells per well.

Example: An overnight culture ($OD_{600} = 2.0$, $\sim 2 \times 10^9$ cells /ml) is made into a 15% glycerol stock by adding 500ul of a 30% glycerol solution to 500ul of the culture. The glycerol stock has a final volume of 1.0 ml and a cell density of $\sim 1 \times 10^9$ cells /ml and is stored at -80°C . To transfer the cells into CloneStable, the glycerol stock is thawed, spun down and resuspended in 500ul of water, giving a cell density of 2×10^9 cells /ml. 20ul is spotted into CloneStable, ($\sim 4 \times 10^7$ cells per well). Note: Viable cell count in glycerol stocks vary depending on frozen storage time and conditions.

2a. Heat Lysis (optional): Bacteria are viable in CloneStable in a dried state for up to a month. If the sample will be used for transformation into a new strain within the first month of storage heat the bacterial suspension at 80°C for 5 minutes prior to application to CloneStable to completely lyse the cells.

3. Add resuspended pellet to CloneStable. Allow the matrix to be hydrated by the aqueous sample for 5-10 minutes.

We recommend spotting a 20ul volume into CloneStable.

4. Mix the well or tube by gentle pipetting.

When pipetting, avoid forming bubbles. Alternatively, mixing can be done using a high speed shaker. Tightly secure the plate onto the shaker. Mix at 800 rpm for 1 minute.

5. Dry the uncovered sample completely at room temperature.

We recommend using a laminar flow hood or drying with a vacuum concentrator (e.g. SpeedVac[®]). Recommended drying times are provided in Tables 1 and 2.

Note: Drying should occur at ambient laboratory temperatures (20°C–25°C) with relative humidity below 40%. We recommend drying under a vacuum if conditions exceed these parameters.

6. Cover samples after drying and store at room temperature.

After complete sample drying, plates should be re-sealed with aluminum foil seals (provided in kit). The aluminum seal will provide an additional moisture barrier to protect the samples. Tubes can be closed using the snap-cap. Place the plates or tubes in a moisture-barrier bag along with the included silica gel desiccant packet.

Samples properly sealed can be stored at ambient temperature with relative humidity below 40%. If these environmental conditions cannot be maintained store samples in a desiccating chamber.

Protocol: Recovery of bacterial DNA

Total bacterial plasmid and genomic DNA from *E. coli* stored in CloneStable can be recovered by the addition of water. Recovered DNA samples are ready for downstream applications without the need for further purification.

Procedure

- 1. Add 20 µl of water or aqueous buffer directly to the dried sample in CloneStable.**

Individual wells in a plate can be opened by puncturing the aluminum foil seal with a razorblade.

Samples may be rehydrated directly with aqueous buffers used for downstream applications.

- 2. Incubate at room temperature for 15 min to allow complete re-hydration.**

After 15 minutes, mix the sample by gently pipetting up and down to resuspend the sample.

The rehydrated sample is now ready for use directly in downstream applications.

- 3. Store unused rehydrated samples at 4°C or room temperature for up to 10 days.**

Rehydrated samples contain CloneStable and can be re-dried without loss of efficient sample stabilization. We do not recommend repeating the rehydration/drying process more than 3 times per sample. Samples can also be stored at -20°C for long term storage.

Protocol: Downstream applications

Bacterial DNA rehydrated from CloneStable samples do not require further purification for the majority of molecular biology applications, and can be used directly without interference or inhibition in downstream applications. For further results and examples, refer to www.biomatrica.com under *Application Notes*.

Should further purification be necessary, samples can be purified using column purification technology from commercially available kits.

Transformation

Samples dried and stored in CloneStable are rehydrated with water and can be added to chemically competent cells. The number of colonies vary based on transformation efficiency. Competent cells with transformation efficiencies of greater than 1×10^6 cfu per μg are recommended.

Transformation example:

E. coli DH10B harboring pUC19 were spotted into CloneStable wells at a density of $\sim 4 \times 10^7$ cells per well (20 μl of a suspension at $\text{OD}_{600} = 2.0$).

- Re-hydrate wells containing *E. coli* stored in CloneStable with 20 μl of water.
- Add 10 μl of the rehydrated sample to 100 μl of chemically competent *E. coli*. Incubate the mixture on ice for 20 minutes.
- Transfer to a 42°C water bath for 45 seconds and back onto ice for 2 minutes.
- Add 900 μl of LB media and incubate the cells on shaker at 37°C for one hour.
- To obtain colonies, spread 100 μl * of the transformation mixture onto LB agar dishes with appropriate selective antibiotic. Incubate plates overnight at 37°C.

*Volume can be adjusted between 20 μl -200 μl depending on transformation efficiency and desired colony count.

PCR Reactions

Re-hydrate wells containing *E. coli* stored in CloneStable with 20 μl of water. Use 1-3 μl of rehydrated DNA samples directly in PCR reactions following manufacturer's guidelines.

Troubleshooting Guide

Dry storage of bacterial DNA in CloneStable has been shown to be very effective for long-term storage of bacterial samples, if attention is paid to ensure complete drying and proper storage conditions (*i.e.* between 20-25°C with relative humidity below 40%). High relative humidity content in the air will reduce the protective properties of CloneStable resulting in sample degradation. Adherence to our recommendations with respect to sample volumes, storage capacity of CloneStable products, and sample drying conditions will ensure consistent and optimal results.

The following answers to frequently asked questions may be helpful in solving any problems that may arise. Scientists at Biomatrix, Inc. Technical Service Department are available to answer questions about the information and protocols in this handbook and molecular biology related applications (see last page for contact information).

Question	Answer	Comments
Can unpurified <i>E. coli</i> be added directly to CloneStable?	Yes. Unpurified bacteria can be directly added to CloneStable.	CloneStable can accommodate <i>E. coli</i> in most common growth media (<i>i.e.</i> LB medium) containing selective antibiotics (<i>e.g.</i> ampicillin, kanamycin).
Can <i>E. coli</i> in glycerol stocks be added directly to CloneStable?	No, glycerol should be washed out and the cells should be resuspended in water.	See page 10 for detailed instructions.
What is the maximum density of cells CloneStable can accommodate?	Both log and stationary phase cells can be stored at densities up to OD ₆₀₀ = 4.0	
Do <i>E. coli</i> dried down on CloneStable survive the dry down process?	Yes. Testing has shown that a fraction of <i>E. coli</i> can remain viable for approximately one month dried down in CloneStable.	To assure there are no live cells, treat heat cells with at 80°C for 5 min prior to adding to CloneStable.
How long does it take for all the cells to lyse after the initial dry down?	The length of time will vary depending on the type and quantity of cells. For large quantities, it can take up to one month for all the cells to lyse completely.	Lysing cells with heat before adding them to CloneStable allows pure DNA recoveries within one month. See protocol on page 8.
What happens to my sample in a humid environment? How does humidity affect storage?	As with any DNA sample there can be degradation with humidity over time. We suggest storage in a dry environment in the foil moisture barrier bags provided with the products.	

Question	Answer	Comments
Can I reduce or increase sample re-hydration times?	Minimum re-hydration time (5 min) is important for full recovery of stored DNA. Maximum re-hydration time should not exceed 1 hour. A shorter rehydration time with mixing can be used; however, there may be a slight reduction in sample recovery.	Re-hydration of 5-10 min is recommended to ensure complete sample recovery. Keep plate or tube covered with lid during re-hydration to avoid contamination and evaporation.
Why is less volume recovered from well than was initially added for re-hydration?	Re-hydration of the dissolvable matrix may cause a 10% reduction in sample volume recovery. This is especially noticeable for smaller volumes (<i>i.e.</i> <20 µl)	<ul style="list-style-type: none"> Minimal loss of recovered sample volume does not affect the stability or performance of DNA or CloneStable in downstream applications. A 10% loss in volume can be adjusted for (<i>e.g.</i> add 11 µl of water to ensure recovery of 10 µl of rehydrated sample).
What happens if I put a sample stored in CloneStable into a freezer (-20°C)? Or at 4°C?	This is not necessary nor is it recommended; however, cold storage does not interfere with CloneStable product performance.	Simply remove samples from cold storage and place at room temperature. Make sure product fully dries prior to room temperature storage. Samples stored with CloneStable may be conveniently and cost-effectively stored and shipped at ambient temperatures.
What is the longest time I can store my samples in CloneStable?	Biomatrixa has in-house real time room temperature storage data for two years and for 8 years under accelerated aging conditions.	
Do I have to purify my sample after rehydration before I use it?	No.	Rehydrated samples can be used directly (<i>i.e.</i> non-purified) in many downstream applications, including transformation and PCR.
How do I remove CloneStable from my sample?	If necessary, use commercially available spin columns (<i>i.e.</i> miniprep spin columns) or standard phenol:chloroform extraction to purify recovered DNA.	Be aware of a certain loss of recoverable DNA after purification procedures.
Does CloneStable work for bacterial strains other than <i>E. coli</i> ?	CloneStable has been optimized for the storage and recovery of total (plasmid and genomic) DNA harbored in <i>E. coli</i> bacterial strains routinely used in life science research (<i>e.g.</i> DH10B, DH5α,	This product is not validated for use with other bacterial strains. Check www.biomatrixa.com for latest news.

Question	Answer	Comments
	Stbl2, etc.).	
Is there a difference between the storage of plasmid and genomic DNA?	No.	
Is there a difference in the storage of plasmids of different size?	No.	
Is there a shelf life to unopened/unused tubes?	We recommend using the product within 24 months of receipt. The color of CloneStable may change over time but this does not impact stability of the medium or DNA storage.	
When several samples are loaded into a new plate, is it possible to protect the unused wells?	In this case it is best to use seal strips available from Excel Scientific (www.excelscientific.com) and follow these procedures: <ul style="list-style-type: none"> • Cut the seal over the columns to be used (a razor blade works well) • Deposit the DNA samples • Air dry samples • Cover wells with seal strips. 	
What is the maximum volume I can put in a well?	We suggest storing up to 20 μ l of sample to ensure complete dry down overnight.	Larger volumes have not been validated and must be dried in a vacuum concentrator. (e.g. speedvac).
Can I store other molecules such as RNA or enzymes/proteins?	No. CloneStable is designed specifically to store bacterial plasmid and genomic DNA.	CloneStable technology is sold for the shipping and storage of plasmid and genomic DNA from bacterial cultures and lysates. Biomatrix, Inc. has other novel products for storage of RNA and is developing products for protein storage.
Can I use a portion of a rehydrated DNA well and redry the remainder for future use? How often can I retrieve DNA from the same well?	We do not recommend multiple uses of the same well due to the possibility of contamination.	We suggest drying down 20ul in each well and using the entire well for each downstream application. If necessary, a well can be hydrated and used partially and redried. We do not recommend this more than 3 times. Cycles of rehydration and drying reduces sample recovery.

Quality Control

Every manufacturing production lot of CloneStable is quality control tested for contamination and functional performance. All products are tested against predetermined specifications to ensure consistent product quality.

Product Use Limitations

CloneStable technology is specifically designed for the stabilization of bacterial DNA samples. No claim or representation is intended for their use on any other biological materials. CloneStable products are for *research use only* and are not intended for use in diagnostic procedures. For optimal performance, products must be used and stored according to manufacturer's guidelines.

Optimal protection of bacterial DNA in CloneStable occurs when samples are prepared and stored at room temperature and in a relative humidity environment below 40%. Please refer to "Sample drying and storage" (pg. 9) for details on preparing and storing DNA under relative humidity conditions exceeding 40%.

Warranty and Satisfaction Guarantee

Biomatrix, Inc. guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for a particular use. Should any of our products fail to perform satisfactorily due to any reason other than misuse, we will replace it free of charge or refund the purchase price, at your request. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a Biomatrix product does not meet your expectations, please contact our Technical Service Department.

Warranties of merchantability or fitness for a particular purpose are expressly disclaimed. Biomatrix's liability shall not exceed the purchase price of the product. Biomatrix shall have no liability for indirect, consequential, or incidental loss or damages from the use, results of use, or inability to use its products. Contact us for a copy of our full limited warranty statement.

Notes

Notes

Technical Assistance

Biomatrix, Inc. takes pride in providing efficient quality technical support. Biomatrix's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of Biomatrix's biostability and storage products. Please contact Biomatrix directly with any questions regarding DNAstable technology, product use, or general matters.

Technical Service Department:

Phone: USA (866) DRY-MTRX or (866) 379-6879

Web: www.biomatrix.com

Email: support@biomatrix.com

Literature Citation

When a procedure utilizing this product is described in a manuscript for publication, kindly refer to it as CloneStable from Biomatrix, Inc.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective eyewear. For more information, please consult appropriate material safety data sheets (MSDS, available online at www.biomatrix.com, in a convenient PDF format).



5627 Oberlin Drive, Suite 120
San Diego, CA 92121