

CloneStab™ Protocol

CloneStab is designed to store live *E. coli* bacteria for short time periods (up to 3 months). CloneStab is an ideal format for shipping *E. coli* containing plasmids of interest.

Kit Components

25 tubes CloneStab (catalog #93121-018)
1 Protocol

Storage

Unused tubes should be stored dry in their original packaging at room temperature (15-25°C). Use within 3 months of purchase date for optimal product performance and sample protection.

Sample Preparation

1. Grow *E. coli* to stationary phase in appropriate selective growth media.
2. Draw a 10 µl sample of the bacterial culture into a sterile pipette tip. (Use of filter tips is recommended to prevent contamination).
3. Open tube and insert pipette tip approximately 8 mm directly into center of CloneStab plug.
4. Simultaneously eject the sample directly into the plug while slowly pulling out the pipette tip. The entire aliquot should be deposited within plug.
Note: If the sample does not eject immediately, gently slide the pipette tip straight up and down a few millimeters while maintaining gentle pressure on the plunger.
5. Close tube cap tightly. The sample is now ready for shipping or storage for up to three months at room temperature.

Sample Recovery – Liquid Media

1. For general cell recovery:
 - a) Add 1 ml selective growth media to the top of the CloneStab plug containing bacteria.
 - b) Vortex tube for 20 sec (the agar plug should be dislodged from the bottom of the tube).
 - c) Incubate overnight while shaking at desired temperature.
Note: Homogenization of the agar plug is not recommended in this case, as agar fragments may interfere with subsequent plasmid isolation.
 - d) The overnight culture can be used directly for plasmid purification, growth on solid media or preparation of glycerol stocks following standard procedures.

2. For greater cell recovery:
 - a) Add 1 ml selective growth media or compatible buffer (antibiotic not necessary) to the top of the CloneStab plug containing bacteria.
Note: The plug may be crushed to facilitate release of bacteria using a sterile toothpick or other utensil.
 - b) Vortex 20 – 30 sec.
 - c) Transfer at least 20 μ l of the recovered bacterial sample to the desired volume of selective growth media.
 - d) Incubate overnight with shaking at desired temperature and proceed with downstream applications using standard protocols.

Sample Recovery – Solid Media

1. Add 0.5 – 1.0 ml selective growth media or compatible buffer (antibiotic not necessary) to the top of the CloneStab plug containing bacteria.
Note: The plug may be crushed to facilitate release of bacteria using a sterile toothpick or other utensil.

2. Vortex 20 – 30 sec (the agar plug should be dislodged from the bottom of the tube).

3. Use 50 – 100 μ l per plate of the resuspended bacterial sample to culture bacteria on solid selective growth media. Alternatively, streak a solid selective agar plate or inoculate a nutrient agar stab directly from the resuspended sample following standard protocols.

Frequently Asked Questions

Situation	Comment	Suggestion
Unused CloneStab color is faded or has a slight change in coloration.	Fading or color change of CloneStab does not affect protective properties	Proceed with sample preparation as directed.
CloneStab plug color is faded or has a slight change in coloration following incubation in growth media.	Fading or color changes after addition of growth media does not affect product performance.	Proceed with sample preparation as directed.
CloneStab plug is released from the tube while piercing the plug and depositing bacterial sample inside.	Bacterial sample should be deposited entirely inside the plug. If the plug is released and the sample is deposited into the bottom of the tube, it is necessary to use a fresh CloneStab tube.	Use a fresh CloneStab tube. Ensure bacterial sample is deposited completely inside CloneStab plug.
Bacterial sample was deposited on top of CloneStab plug.	Recovery of live bacteria and plasmid DNA will be significantly affected if the bacteria are not deposited completely inside the CloneStab plug.	Use a fresh CloneStab tube. Ensure bacterial sample is deposited completely inside CloneStab plug.
Fragments of CloneStab plug remain in the tube after overnight incubation in liquid growth media.	Residual CloneStab fragments should be removed if liquid culture is to be used in plasmid purification protocols/applications (i.e. miniprep).	Spin the tube gently to pellet residual CloneStab fragments.
CloneStab plug breaks into fragments after vortexing prior to incubation in growth media.	Fragmenting of the CloneStab plug during vortexing is normal and will not affect product performance or downstream applications. Bacteria are released more efficiently by gently vortexing the CloneStab plug,	Residual CloneStab fragments should be removed if liquid culture is to be used in plasmid purification protocols/applications (i.e. miniprep). Spin the tube gently to pellet residual CloneStab fragments.
Bacteria did not grow following overnight incubation in liquid growth media.	More bacteria may need to be released from CloneStab plug. Proper aeration of liquid bacterial culture may be needed.	Vortex vigorously or crush the plug to release bacterial stored in CloneStab plug. Increase the volume of selective media used to grow overnight culture to ensure proper aeration bacterial cells.
Inoculated CloneStab plug was incubated overnight at 37°C prior to storage or sample shipment at room temperature.	Identical results were obtained (i.e. recovery of live bacteria and subsequent plasmid purification) with or without overnight incubation at 37°C prior to storage or shipment.	Incubation of inoculated CloneStab plug at 37°C for longer than 18 hours (i.e. overnight) is not recommended.