

Supercoil-It™

Catalog # S-101

Lot: 1

Contents Supplied:

- (1) 100 ul Supercoil-It enzyme reagent
- (2) 2.5 ml 20X Supercoil-It Buffer

Storage: Store both enzyme reagent and buffer at -60 °C or below. Repeated freeze-thaw cycles have no effect on enzyme reagent or buffer.

Description: The Supercoil-It plasmid purification system is designed to convert any plasmid preparation to a preparation containing virtually 100% supercoiled plasmid, regardless of the initial percentage of open circular plasmid, plasmid size, plasmid sequence, and plasmid source. This is accomplished by enzymatically converting virtually all the open circular plasmid to supercoiled plasmid. The Supercoil-It plasmid purification system is favorably used as an added step in any plasmid purification method, in order to achieve a preparation having virtually 100% of plasmid in supercoiled form. Supercoil-It does not contain topoisomerase I. Therefore, the plasmid product will be highly supercoiled plasmid, which is supercoiled to its thermodynamic limit. The kit contains sufficient reagents for purifying 150 mg of plasmid. Supercoil-It is **NOT** for use in purifying plasmid for human therapy.

Directions for Use:

- (1) Prepare a plasmid solution having a plasmid concentration not exceeding 3.2 ug/ul.
- (2) Add 1/19 volume of 20X Supercoil-It Buffer to the plasmid solution, mix thoroughly.
- (3) Add 2 ul Supercoil-It enzyme reagent per milliliter of the resulting volume, mix thoroughly, incubate at 37 degrees for 2 hours.
- (4) Recover the plasmid after the incubation, by further purification to remove enzyme reagent and buffer (optional step).

Preparing the Plasmid Solution:

The plasmid solution contains purified or semi-purified plasmid at a concentration up to 3.2 ug/ul, preferably dissolved in TE buffer (pH 7.5 to 8.0). Plasmid solutions containing phosphate buffer are not recommended. To maximize the amount of plasmid purified using the kit, use the highest plasmid concentration of 3.2 ug/ul in the plasmid solution.

The Supercoil-It system is based on purification by enzymatic reactions. These enzymatic reactions are more reliable when using a purified (or semi-purified) source of plasmid. Therefore, the starting plasmid solution is preferably obtained by a purification method which removes most of the salts, protein, and RNA from the host cells (as such impurities may hinder the enzymatic reactions). Although any plasmid purification method may be used, the recommended method is column purification. Numerous column based purification kits are commercially available, e.g. Qiagen, Clontech, Promega, Ependorf, Sigma, and Qbiogene. If plasmid is eluted from a column in high salt, the eluted plasmid should be concentrated by alcohol precipitation and dissolved in TE buffer at a final plasmid concentration up to 3.2 ug/ul. If a plasmid purification method is not column based, the purification method should effectively reduce salt, protein, and RNA contamination to ensure an efficient enzymatic reaction. For example, the standard Maniatis alkaline lysis method may be used to make the plasmid solution (alkaline lysis, then phenol:CHCl₃ extraction, then alcohol precipitation, then dissolve pellet in TE buffer + RNase, Maniatis, Molecular Cloning, second edition, Cold Spring Harbor Laboratory Press, 1989). The user is strongly advised to determine initially on a small scale whether the Supercoil-It reaction is efficient for a given purification method, before proceeding to a large

scale reaction. If the Supercoil-It reaction is not complete in two hours, the incubation may be extended for an additional two hours.

Plasmid Recovery:

After the Supercoil-It enzyme incubation, plasmid may optionally be recovered using any desired purification method. This purification step can remove the enzymes and nucleotides used in the reaction. One recommended approach is to further purify the plasmid using the same purification column which was used to make the plasmid solution. For columns such as Qiagen and Macherey-Nagel, the incubated plasmid can be loaded onto the same anion exchange cartridge, washed, and eluted using the manufacturers recommendations. Make sure that the cartridge has been pre-cleaned prior to re-use by washing with elution buffer, then re-equilibrating the column in binding buffer. Other methods for recovering the plasmid may be used. For example, the incubated solution may be extracted with phenol:CHCl₃, extracted with CHCl₃, followed by plasmid precipitation using alcohol and sodium acetate.

Note on Dimeric Plasmid

Dimeric plasmid exists in virtually all plasmid preparations, because E. coli hosts convert some of the monomeric plasmid to dimeric plasmid. Dimeric plasmid is a single plasmid molecule, created by covalent recombination of two monomeric plasmids in E. coli. Dimeric plasmid replicates in the E. coli host, just as the monomeric plasmid replicates. Usually, dimeric plasmid represents a very small percentage of the total plasmid. This percentage varies for different hosts and for different plasmids.

After purification using Supercoil-It, a small amount of an upper band may be visible on agarose gel. This upper band is dimeric supercoiled plasmid, not open circular plasmid. Dimeric supercoiled plasmid migrates very slightly above (i.e. more slowly than) open circular plasmid on agarose gel. Because gel migration of supercoiled dimeric plasmid is nearly identical to open circular monomeric plasmid, it is sometimes falsely concluded to be open circular plasmid. Supercoil-It will not remove the dimeric plasmid. Instead, Supercoil-It will convert any open circular dimeric plasmid to supercoiled dimeric plasmid (in the same manner as it converts the open circular monomeric plasmid to supercoiled monomeric plasmid). Therefore, an increase in the amount of supercoiled dimeric plasmid may be observed after the reaction. Supercoil-It does not create and does not remove any dimeric plasmid. Supercoil-It does not create any significant catemeric (chain linked) plasmid. In some applications, Supercoil-It may actually decatenate any catemeric plasmid, which may be present in a plasmid solution prior to the incubation.

Notes on Use:

- (a) Incubation with Supercoil-It enzyme reagent for longer than 4 hours is not recommended. Such over-incubation may result in plasmid preparations having poorly supercoiled plasmid, relaxed covalently closed circular plasmid, and/or degraded plasmid forms. An initial small scale reaction is strongly recommended to ensure efficacy, prior to a large scale reaction.
- (b) In another application, Supercoil-It may be used to convert open circular recombinant plasmid formed in a ligation reaction (linearized vector + insert DNA) to supercoiled form, in order to potentially increase the number of recombinant transformants. If the linearized cloning vector has 5'-phosphate termini, Supercoil-It will re-circularize the cloning vector.
- (c) Supercoil-It is **NOT** for use in purifying plasmid for human therapy.

Legal Disclaimer: Customer agrees that the Supercoil-It enzyme reagent is experimental in nature, and the effect of this reagent on the biological activity of plasmid for any particular application is unknown and cannot be guaranteed. Bayou Biolabs makes no guarantee on bioactivity of plasmid obtained using this reagent. Because the customer provides the plasmid solution used in the Supercoil-It reaction, Bayou

Biolabs makes no guarantee on the conversion reaction efficiency of open circular plasmid to supercoiled plasmid. Additionally, Bayou Biolabs makes no guarantee that the Supercoil-It reaction will not result in an inferior plasmid preparation, such as a preparation having poorly supercoiled plasmid, having relaxed covalently closed circular plasmid, having increased multimeric forms, and/or having degraded plasmid forms. Customer agrees that Bayou Biolabs shall not in any event be liable for any loss, claim, or damages of any kind (including direct, incidental, consequential, punitive), which may arise from use of this reagent and which may arise from use of any plasmid (or related product) prepared by this reagent. Customer agrees that this reagent is NOT for use in humans. Customer agrees that this reagent is NOT for use in preparing plasmid (or related product) for human use in any conceivable manner. Customer agrees that this reagent is NOT for use in preparing plasmid (or related product), which is subsequently used for generating virus for human use (e.g. adenovirus, retrovirus, etc). Customer agrees that this reagent is for research purposes only.

Supercoil-It™ is patent protected, US patent 7510856, and is a trademark of Edward Hyman.
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BAYOU BIOLABS, LLC

4724 Hessmer Avenue, Metairie, LA 70002

phone 504-723-1703

Email: support@bayoubiolabs.com

www.bayoubiolabs.com