Exonuclease-It™

Catalog # X-101
Lot: 1
Contents Supplied:
(1) 500 ul Exonuclease-It™ enzyme reagent
(2) 1.4 ml 40X Exonuclease-It™ Buffer

Storage: Store both enzyme reagent and buffer at -20 °C or below. Repeated freeze-thaw cycles have no effect on enzyme reagent or buffer.

Description: The Exonuclease-It™ plasmid purification system is designed to selectively digest linear chromosomal DNA contamination in a plasmid preparation, without significantly degrading any supercoiled plasmid (or any covalently closed circular plasmid). Exonuclease-It™ will also degrade open circular plasmid and circular single stranded DNA. The Exonuclease-It™ enzyme reagent contains a proprietary mixture of exonucleases to accomplish this task. In a typical 6 hour incubation, Exonuclease-It™ will reduce the amount of chromosomal DNA contamination in a plasmid preparation by 98 percent. The by-products of the exonuclease digestion are deoxynucleoside monophosphates. The Exonuclease-It™ plasmid purification system is ideally used as an added step in any plasmid purification method, in order to thoroughly remove chromosomal DNA. This kit contains sufficient reagents for purifying 150 mg of plasmid. Exonuclease-It™ is **NOT** for use in purifying plasmid for human therapy.

Directions for Use:
(1) Prepare a plasmid solution containing a plasmid concentration not exceeding 3.2 ug/ul.
(2) Add 1/40 volume of 40X Exonuclease-It™ Buffer to the plasmid solution, mix thoroughly.
(3) Add 10 ul Exonuclease-It™ enzyme reagent per milliliter of the resulting volume, mix thoroughly, incubate at 37 degrees for the desired incubation time (usually 2 – 6 hours).
(4) Purify the plasmid to remove the enzymes and dNMP digestion by-products (optional step).

Chromosomal DNA digestion:
Results from a typical incubation are shown below using real time PCR quantitation of E. coli chromosomal DNA in a plasmid preparation. User results may vary.

incubation time	percent chromosomal DNA remaining
0	100 %
2 hrs	8.9 %
4 hrs	3.2 %
6 hrs	2.1 %
8 hrs	2.0 %

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 Exonuclease-It™ system is based on purification by exonuclease 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:$CHCl_3$ 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 Exonuclease-It™ reaction is effective for a given purification method, before proceeding to a large scale reaction.

Plasmid Purification after the Incubation:
After the Exonuclease-It™ incubation, plasmid may be purified to remove the enzymes and nucleotide by-products in the reaction. This may be accomplished by using commerically available anion exchange columns (e.g. Qiagen, Macherey-Nagel) or other purification kits. The same anion exchange column which was used to prepare the plasmid solution may be used to purify plasmid after the Exonuclease-It™ reaction. In this case, make sure that the anion exchange cartridge has been cleaned thoroughly prior to re-use by washing thoroughly 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:$CHCl_3$, extracted with $CHCl_3$, followed by plasmid precipitation using alcohol and sodium acetate.

Note on Open Circular Plasmid
Exonuclease-It™ will degrade open circular plasmid to the (+) and (-) strands of single stranded circular DNA. These (+) and (-) circular strands spontaneously anneal to form a duplex structure resistant to further Exonuclease-It™ digestion. If sufficient open circular plasmid is present initially in the plasmid solution, this duplex structure may be visible on gel electrophoresis as a band migrating just above (slower than) the supercoiled plasmid band.
If a plasmid preparation contains a large amount of open circular plasmid, loss of this open circular plasmid in the Exonuclease-It™ reaction may be unacceptable. In this case, we recommend using Supercoil-It™ prior to using Exonuclease-It™. Supercoil-It™ will convert virtually all open circular plasmid to supercoiled plasmid. The will prevent the loss of this plasmid in the subsequent Exonuclease-It™ reaction.

Use of Exonuclease-It™ after Supercoil-It™
Exonuclease-It™ may be used after Supercoil-It™ incubation. The enzymes in the Supercoil-It™ reaction will strongly hinder the performance of the Exonuclease-It™ reaction, and should be inactivated or removed prior to the Exonuclease-It™ reaction. The Supercoil-It™ enzymes can be removed by phenol:$CHCl_3$ extraction, followed by alcohol precipitation of the plasmid. Alternatively, the Supercoil-It™ enzymes can be removed by column purifying the plasmid, e.g. using a Qiagen or Macherey-Nagel anion exchange column.

Notes on Use:
(a) Exonuclease-It™ may be used in any application to selectively digest linear DNA (single stranded or double stranded) without significantly degrading covalently closed circular plasmid. For example, a mixture of linear DNA and covalently closed circular DNA may be generated by

a ligation reaction. Exonuclease-It™ can selectively digest the linear vector DNA prior to cell transformation, in order to reduce background transformants.
(b) Incubation with Exonuclease-It™ enzyme reagent for longer than 8 hours is not recommended. Such over-incubation may result in unacceptable digestion of supercoiled plasmid. An initial small scale reaction is recommended to ensure efficacy, prior to a large scale reaction.
(c) Exonuclease-It™ is **NOT** for use in purifying plasmid, or any other DNA, for human therapy.

Disclaimer: Customer agrees that the Exonuclease-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. Because the customer provides the plasmid solution used in the Exonuclease-It™ reaction, Bayou Biolabs makes no guarantee on the amount of chromosomal DNA digested, nor the amount of plasmid loss in the reaction. Customers are urged to perform the reaction on a small scale prior to large scale reaction, to ensure efficacy. 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 DNA or any 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 DNA or any related product) for human use in any conceivable manner. Customer agrees that this reagent is NOT for use in preparing plasmid (or DNA 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.

Exonuclease-It™ and Supercoil-It™ are a trademarks of Edward David Hyman.
Version 1 of this instruction sheet, December 21, 2006.

BAYOU BIOLABS, LLC

Email: support@bayoubiolabs.com

www.bayoubiolabs.com