

Plasmid DNA Purification Services Request Form

Date: _____ P.O. Number: _____

Investigator Name: _____

Address: _____

Phone: _____ Fax: _____ Email: _____

Plasmid Information:

Starting material: (Check one) DNA (requires at least 1 µg as liquid, or 2-3 µg on filter paper) Con.: _____ Colony on plate Glycerol stock Liquid culture Other _____

Plasmid Name: _____ Size: _____

Host strain (if provided as bacteria): _____

Antibiotic: _____ (Amp, Kan, chloramphenicol provided at no charge) Concentration: _____

Plasmid copy per bacterium: High (>10 copies, higher than 1 mg/liter), Low (< 10 copies, lower than 1 mg/liter), Unknown

Preferred host (if provided as DNA for transformation): (Circle one) DH5α, Provided: _____

Diagnostic enzyme(s): (Extra charge will be applied for uncommon enzymes. Restriction analysis is not included in Express grade services.)

1. _____ Expected fragments (in kb): _____

 Additional information attached.Plasmid DNA will be used for : Cloning/probe synthesis/bacteria transformation Cell transfection Animal studies Others: _____

Production Information:

Type of production: Standard Quality Transfection Quality Mega Size Plasmid BAC Plasmid (Check one) BAC Plasmid for animal studies (plus endotoxin removal) Express Express Plus

Scale: (Circle one) Express scale (250 ml), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 liters, other _____

DNA to be dissolved in: (Circle one) TE (standard), water (for DNA sequencing and electroporation).

Special instruction: _____

Lab use only: Material Received On: _____ By: (Circle one) Direct pick-up, Mail

Production No. _____ Date of completion: _____

Comments: _____



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Frequently Asked Questions

I. How much starting materials do I need to send you for the purification service?

If you send us bacteria, you can send us a plate, liquid culture in a tube, glycerol stock, or a slab. Please wrap the plate or tube with parafilm so it will not open during shipping. If you send it by mail, we recommend wrapping them with additional bubble wrap so they will not be crushed by automatic sorting machine.

If you send us DNA, we will need **at least 1 µg as liquid, or 2 - 3 µg on filter paper** for transformation.

II. We need specific amount of plasmid DNA. How do you charge for the plasmid DNA purification services?

Since the yield of plasmid vary depending on the nature of the plasmid. Instead of charging the services by quantity such as mg, we offer the services by liter production. In general, for a high copy plasmid such as pCDNA based plasmid, the average yield is between 2-4 mg per liter. We offer a guarantee yield of 1 mg per liter for high copy plasmid, 0.3 mg per liter for low copy plasmid such as BAC. If one needs specific amount of plasmid DNA, we can do a one-liter production first to get an estimate of yield of a particular plasmid, then we will send you a quote for the production of the specific amount of DNA requested.

III. What kit do you use for the transfection grade plasmid purification service?

The kit we used for the service is Qiagen Endofree plasmid purification kit. For more information, please check their web site. <http://www1.qiagen.com/products/Plasmid/QIAGENPlasmidPurificationSystem/EndoFreePlasmidMegaKit.aspx>

IV. What is the level of endotoxin in the plasmid DNA purified from your transfection grade purification service?

According to Qiagen, the endo-free kit can reduce endotoxin level to less than 0.1 EU/ug DNA (or 100 EU/mg DNA). We had independently verified their data using an independent testing company and showed that our preps contain about 0.1 EU/ug DNA (or 100 EU/mg DNA) or less of endotoxin.

V. What is the difference on endotoxin level between standard and transfection grade plasmid DNA preparation?

Using an independent testing company, we found the standard grade plasmid DNA we prepared have about 5 - 10 EU/ug DNA (or 5000 - 10,000 EU/mg DNA) and the transfection grade plasmid DNA we prepared have about 0.1 EU/ug DNA (or 100 EU/mg DNA).

VI. What test kit is used to detect endotoxin?

The most common test kit used to detect endotoxin is the LAL test sold by Cambrex Bioscience. We don't know what test Qiagen uses. The testing company we used uses the LAL test kit from Cambrex Bioscience. For more information on the LAL test, please check their web site. <http://www.cambrex.com/Content/bioscience/CatNav.oid.665.prodoid.KineticOCL>

VII. Do you do endotoxin test on every DNA purified by the transfection grade purification service?

We purify plasmid DNA according to the procedures of the Qiagen's Endofree kit and don't do endotoxin test on every transfection grade plasmid DNA we purified. However, if our client needs us to perform the endotoxin test on the transfection grade DNA we prepared, we can send it out to an independent testing company to perform the test at extra charge. As part of our quality control, we do test periodically using an independent testing company and found that our transfection grade plasmid DNA matched to the specification of Qiagen's Endofree kit's claim, that is, less than 0.1 EU/ug DNA (or 100 EU/mg DNA).

VIII. What is the meaning of EU (endotoxin unit)?

According to Cambrex Bioscience, the maker of the LAL test kit, depending on the source of the endotoxin, the conversion from endotoxin units to nanograms will vary. The FDA initially defined the Endotoxin Unit (EU) as the endotoxin activity of 0.2 ng of Reference Endotoxin Standard, EC-2 or 5 EU/ng. To convert the current FDA RSE, EC-6 from EU's into ng, the conversion is 10 EU/ng. Previous versions of the BET chapter <85> in USP stated a suitable CSE has potency between 2 and 50 EU/ng.

IX. What is the acceptable level of endotoxin in plasmid DNA used in cell transfection and animal experiments?

There is no general standard for cell transfection experiment because different cells reponse to endotoxin differently. For example, cells from neuronal region are more sensitive to endotoxin while fibroblast are less sensitive. It is commonly agreed that a preparation of 0.1 EU/ug DNA (or 100 EU/mg of DNA) or less should be used for both endotoxin sensitive cells and animal experiments. Most of the commercial endofree plasmid purification kits from companies such as Qiagen, Promega, Sigma are able to reduce endotoxin in a plasmid prep to about 0.1 - 0.5 EU/ug DNA (or 100-500 EU/mg DNA).

X. What is the best way to ship starting materials for your purification services?

Within USA, one can send us DNA as liquid or on filter paper, glycerol stock, colonies on plate, liquid culture or bacteria slab. For our international clients, it is better to send us DNA so there is no dry ice or cold pack involved in the shipment to save shipping cost. We provided glycerol stock at no extra charge when we ship back the purified plasmid DNA to our client. Glycerol stock requires dry ice shipment. If one does not need glycerol stock back, please notify us and we will ship back the DNA in cold pack to reduce shipping cost.



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