Product Name : Long ssDNA Gel Extraction Kit for 3kb

Code No. : DS640 *This product is for research use only*

Kit Components: 25 reactions

Components	Contents	Size
Crystal Violet Solution	Crystal Violet 4mg/ml (2,500 x).	5 ml
Gel-Dissolving Buffer	It contains guanidine thiocyanate as chaotropic salts.	45 ml
Wash Buffer 1	Tris-base buffer (Add 45 ml of 100 % ethanol to the Wash Buffer before use.)	8 ml
Wash Buffer 2	Tris-base buffer (Add 45 ml of 100 % ethanol to the Wash Buffer before use.)	11 ml
Elution Buffer	10 mM Tris-HCl, pH 8.0.	5 ml
Spin Column		25 pieces
Collection Tube		25 pieces

- Ethanol and isopropanol are required for the product.
- If precipitated material has formed in Gel-Dissolving Buffer, heat to dissolve at 37℃.
- Heat block are required for 50-70℃ incubation.
- Denaturing Gel-Loading Buffer is required for the agarose gel electrophoresis of long ssDNA. It is not included in the kit. It can be purchased individually from BioDynamics Laboratory Inc. (see "Related Products" on page 4).

Introduction: The Long ssDNA Gel Extraction Kit is a highly-specialized kit for long ssDNA purification from agarose gel. The spin column, buffer formulations and purification protocol are optimized for high recovery yields and high purity of long ssDNA. Long ssDNA purified with the kit is high-quality, with low mechanical degradation and without ultraviolet light-induced damage from the purification process. The purified long ssDNA is suitable for molecular biology and biotechnology applications.

The Long ssDNA Preparation Kit can be universally used for purification and clean-up of long ssDNA.

Features and Specifications:

- Optimized for long ssDNA.
- High recovery yield (typically 75-90%).
- High purity.
- Low mechanical degradation.
- No UV light-induced DNA damage.
- Direct monitoring of the migration of long ssDNA blue bands in the gel during electrophoresis.
- Excision of long ssDNA under ambient light.
- Guanidine thiocyanate is used as chaotropic salts. NaI is not used.*1
- ssDNA size for excision: 500 - 3,000 bases. *2
- Binding capacity on a Spin Column for ssDNA binding is up to 5µg.
- Elution volume: ≥ 15 µl.

*1 Residual NaI may be difficult to remove, and reduces the efficiency of downstream enzymatic reactions.

*2 200 bases of long ssDNA also can be extracted using the kit, but the recovery yield is not so high (about 40-45%).

Storage conditions: Stable at 15 to 25℃ for 24 months from the date of receipt.

Caution! Wear gloves and protective clothing while handling the Crystal Violet.

Protocol of DNA excision and purification:

STEP 1: Preparation of agarose gel containing Crystal Violet

An example of this is given for making 100 ml of a 1.2% agarose gel.

1. Add 1.2 g of agarose powder to 100 ml of 1×TAE buffer and mix them.
2. Dissolve the agarose in the microwave.
3. Add 40µl of Crystal Violet Solution to the agarose (100 ml) and mix them by swirling.
4. Pour the agarose into a mold and set a comb.* Allow the gel to harden.

* It is recommended to make thick gel with deep wells so as not to cause the overflow of a loaded sample in a well, resulting in contamination.

STEP 2: Agarose gel electrophoresis and excision

A simple way is shown in the following example.

1. Pre-chill 1xTAE running buffer containing Crystal Violet (add 40µl Crystal Violet /100ml 1xTAE) on ice or in a refrigerator. *1
2. Put the horizontal gel electrophoresis apparatus on ice in an ice bucket in a draft chamber. *2, *3, *4
3. Pour pre-chilled 1xTAE running buffer containing Crystal Violet into the electrophoresis apparatus. *5
4. Put the agarose gel into the electrophoresis apparatus.
5. Prepare loading samples by mixing the long ssDNAs sample (e.g. the nicked plasmid) and 3 volumes of the Denaturing Gel-Loading Buffer. *6, *7, *8
6. Heat the mixture at 70°C for 5 min, then chill on ice for 1 min. Subject the mixture to agarose gel electrophoresis. *9, *10
7. Run the gel at 100 V at a low temperature (less than 20℃). *2, *3, *4
8. Monitor the long ssDNA blue bands moving in the gel during electrophoresis (Figure 1). Stop the electrophoresis after the bands are sufficiently resolved. *11, *12
9. Excise the long ssDNA band of interest from the agarose gel with a scalpel. *13, *14

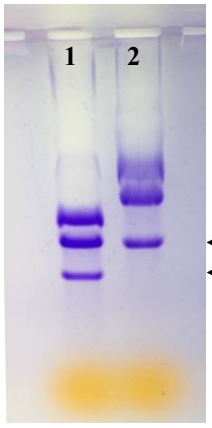

Figure 1. Agarose gel electrophoresis using the gel containing Crystal Violet.
1.2% agarose gel in 1x TAE, 5.5cm x 6.0 cm. The loading volume of each sample is 24 µl.
Lane 1: pLSODN-1 (1.5 Kb Fragment), double-nicked and denatured *15 12 µg
Lane 2: pLSODN-3 (3 Kb Fragment), double-nicked and denatured *15 12 µg

*1 1xTAE with Crystal Violet (add 40 µl Crystal Violet /100ml TAE) should be used as running buffer.
*2 For the safe use of Crystal Violet, electrophoresis with Crystal Violet should be carried out in a draft chamber.

Don't do electrophoresis with Crystal Violet or ethidium bromide in a closed space (especially in a cold room). These dyes diffuse into the air with the water vapor or bubbles generated by electrolysis during electrophoresis.

*3 Electrophoresis at a low temperature is recommended (less than 20℃). It gives a better resolution and strong Crystal Violet staining. If the temperature of gel and running buffer exceeds 25℃, Crystal Violet staining of long ssDNA becomes weak. If so, the gel can be restained by gentle shaking in a dilute Crystal Violet solution (40 µl of Crystal Violet Solution / 100 ml of water) for 0.5 to 1 hour after electrophoresis.

*4 To keep the gel temperature low during electrophoresis in a draft chamber, we recommend placing the electrophoresis apparatus on ice in an ice bucket.

*5 At least, use a pre-chilled 1xTAE running buffer on ice to keep a low temperature.

*6 Denaturing Gel-Loading Buffer is not included in the kit. It can be purchased individually from BioDynamics Laboratory Inc. (see "Related Product "on page 4)

*7 **Desalting of the long ssDNAs sample (or the nicked plasmid) solution in advance is important** for agarose gel electrophoresis. If the long ssDNAs sample (or the nicked plasmid) solution contains excessive salt and Mg^{+2}, it adversely affects denaturation, resulting in giving poor bands and low recovery yields of the long ssDNA of interest. If your long ssDNA band is extremely weak, first you should consider the existence of residual salt in the long ssDNAs sample (or the nicked plasmid) solution. We recommend removing the salt in the long ssDNAs sample through ethanol precipitation. Importantly, wash with 70% ethanol twice, vortex vigorously during washing. In ethanol precipitation and washing operations, the supernatant is usually simply removed after centrifugation, but to ensure thorough desalting, it should be further centrifuged again afterward to collect residual liquid adhering to the wall surface and remove it thoroughly with a micropipette.

*8 To get a highly purified long ssDNA, the long ssDNA of interest should be loaded greater than 1µg/band. **At the same time, the concentration of the nicked plasmid in the loading sample should be under 0.5 µg/µl** after mixing with the Denaturing Gel-Loading Buffer.

*9 The heating and cooling procedure of the long ssDNAs sample (e.g. the nicked plasmid) mixture should be carried out just before loading into wells of the gel.

*10 Do not load a sample too much in a well because it may overflow and cause contamination. It is recommended to make deep wells with thick gel.

*11 **Long ssDNA bands moving in Crystal Violet-containing gel can be directly seen under ambient light in real time during electrophoresis.** The electrophoresis can be stopped as soon as the bands are sufficiently resolved.

*12 Because crystal violet (positively-charged molecule) migrates toward the cathode, the dye concentration of the running buffer and the gel on the anode side gradually decreases. Check the dye concentration and if needed, add dye into the running buffer on the anode side during electrophoresis (e.g. 25 µl of Crystal Violet Solution is added to 250ml running buffer on the anode side every 30 min at 100V constant voltage).

*13 The long ssDNA band can be visible as a blue band and can be easily excised from the agarose gel on the bench.

*14 Minimize the size of the gel slice by removing extra agarose.

*15 The pLSODN-1 (1.5 kb Fragment) was constructed by inserting a 1.5 kb fragment of DNA between the Nt.BspQI and the Nb.BsrDI sites of pLSODN-1 (see the Data Sheet of the Long ssDNA Preparation Kit #615 & #625). The pLSODN-3 (3 kb Fragment) was also constructed in a similar way. The double-nicked plasmids were obtained by double digestion with Nt.BspQI and Nb.BsrDI. The double-nicked pLSODN-1 (1.5 kb Fragment) gives three linear ssDNAs (1,500 bases, 3,251 bases and 4,751 bases) and one circular ssDNA (4,751 bases). Similarly, the double-nicked pLSODN-3 (3 kb Fragment) gives three linear ssDNAs (3,000 bases, 6,487 bases and 9,487 bases) and one circular ssDNA (9,487 bases).

STEP 3: DNA extraction and purification

Before start:

- Add 45 ml of 100 % ethanol to Wash Buffer 1 and Wash Buffer 2 bottles, respectively.
- Isopropanol is required in the step.
- All centrifugation steps should be carried out at 16,000×g (around **13,000 rpm** in a conventional microcentrifuge) at room temperature (20℃-25℃). Centrifugation at lower temperature might affect long ssDNA yield.
- This kit can also be used for long ssDNA clean-up from enzymatic reactions. For this purpose, add 3 volumes of Gel-Dissolving Buffer and 1 volume of isopropanol to the reaction, mix well, and proceed with step 5 of the protocol.

1. Transfer the gel slice to a 1.5 ml microcentrifuge tube and weight the gel slice.
2. Add 3 volumes of Gel-Dissolving Buffer.
3. Incubate the tube at 50℃, vortexing periodically until the gel slice is completely dissolved for 10-15 min. *1
4. Add one gel volume of isopropanol to the dissolved gel and mix well.
5. Insert a Spin Column into a Collection Tube.
6. Load the sample to the Spin Column and centrifuge for 1 min. Discard the flow-through in the Collection Tube with a 1ml pipette tip. *2, *3, *4
7. Centrifuge the Spin Column again for 1 min. Remove the residual flow-through completely with a 10 µl or a 100 µl pipette tip. *5
8. Add 500 µl of Wash Buffer 1 to the Spin Column and centrifuge for 1 min. Discard the flow-through in the Collection Tube with a 1ml pipette tip (1st Wash). *3, *4
9. Repeat wash with Wash Buffer 1 (Step 8, 2nd Wash). *3, *4
10. Add 500 µl of Wash Buffer 1 to the Spin Column, close the cap tightly and vortex for 5 seconds to wash the whole inner wall of the Spin Column (3rd Wash).
11. Centrifuge for 1 min. Keep the flow-through in the Collection Tube and vortex for 5 seconds to wash both the outer wall of the Spin Column and the inner wall of the Collection Tube.
12. Centrifuge for 1 min. Discard the flow-through in the Collection Tube using a 1ml pipette tip. *3, *4
13. Add 500 µl of Wash Buffer 2 to the Spin Column and centrifuge for 1 min. Discard the flow-through in the Collection Tube with 1ml pipette tip (4th Wash). *3, *4
14. Centrifuge the Spin Column again for 1 min to remove residual Wash Buffer 2 completely.
15. Transfer the Spin Column into a new microcentrifuge tube.
16. Add 15-40 µl of Elution Buffer onto the Spin Column and incubate it at 70℃ for 5 min. *6
17. Directly Centrifuge the Spin Column for 1 min to elute long ssDNA. *7, *8, *9

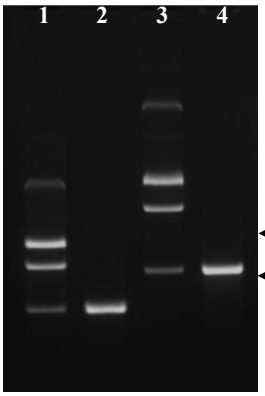

Figure 2. Agarose gel electrophoresis of purified long ssDNAs.
1.2% agarose gel in 1x TAE. 5.5cm x 6.0 cm.
Lane 1: pLSODN-1 (1.5 kb Fragment), double-nicked and denatured 800 ng
Lane 2: a long ssDNA purified from pLSODN-1 (1.5 kb Fragment) 400 ng
Lane 3: pLSODN-3 (3 kb Fragment), double-nicked and denatured 800 ng
Lane 4: a long ssDNA purified from pLSODN-3 (3 kb Fragment) 400 ng

*1 Dissolve the agarose completely. If not, it might decrease the recovery yield of long ssDNA and cause contamination of agar or buffers.

*2 If the volume of dissolved sample exceeds 500 µl, the loading of the sample onto the column should be performed in multiple rounds not to exceed 500 µl.

*3 Don't flip the Collection Tube to discard the flow-through. It contaminates the edge and upper inner wall of the Collection Tube. Use a 1 ml pipette tip to discard the flow-through.

*4 Be careful to ensure the column tip does not contact column flow-through. If possible, we recommend using a new Collection Tube at several washing steps to avoid contamination. However, spare collection tubes are not included in the kit. They can be purchased from many companies (e.g. Corning Axygen #MCT-200-NC).

*5 Don't wash the Spin Column with Gel-Dissolving Buffer only.

*6 Typical elution volumes are 15 µl-40 µl. Water can be used to elute the long ssDNA. The average elution volume is 38 µl from 40 µl Elution Buffer volume, and 13 µl from 15 µl.

*7 The total yield of ssDNA recovery from a 12 µg of a nicked plasmid pLSODN-1(1.5 kb Fragment) or pLSODN-3 (3 kb Fragment) was about 1.7 µg (100% recovery is 2 µg) using one column of the Long ssDNA Gel Extraction Kit for 3 kb with 40 µl Elution Buffer.

*8 Generally, samples eluted from silica based spin columns contain a small amount of silica matrix. If necessary, it could be easily removed by spin down (e.g. 20,000×g for 10 min) or filtration using filter spin column (e.g. Merck Ultrafree-MC GV 0.22 µm).

*9 When quantifying the amount of lssDNA using a spectrophotometer, it is recommended to remove contaminated silica matrix by spinning down and collecting the supernatant before measurement. Presence of silica matrix can cause UV scattering, resulting in a uniform elevation of the baseline between 220nm to 320nm, and potentially leading to an overestimation of lssDNA quantity.

Related Products:

DS611	Denaturing Gel-Loading Buffer	1 ml x 5	(500 loadings)
DS612	Denaturing Gel-Loading Buffer	1 ml x 2	(200 loadings)
DS615	Long ssDNA Preparation Kit for 1.5 kb		
DS625	Long ssDNA Preparation Kit for 3.0 kb		
DS635	Long ssDNA Preparation Kit for 10 kb		
DS650	Long ssDNA Gel Extraction Kit for 10 kb		(25 preps)

BioDynamics Laboratory Inc.
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