Top-Bio s.r.o.

Tel: +420 603 476 934
E-mail: top-bio@top-bio.cz
www.top-bio.cz

Column DNA Lego Kit

UNIVERSAL KITS FOR RAPID ISOLATION OF PURE DNA
(Catalogue number D201 + D 202) rev. 01/2022

Description

Column DNA Lego Kit is a universal modular system (Lego-like) for pure DNA isolation from various sources. The method is based on capacity of silica surfaces to bind DNA in the presence of chaotropic *agents (Proc. Natl. Acad. Sci USA, 76: 615-619, 1979)*. When compared to other methods, DNA isolation with Column DNA Lego Kit is faster (does not require ultracentrifugation, ethanol precipitation and/or drying), safer (does not require phenol extraction and/or use of ethidium bromide) and purer (no contamination with proteins, RNA, and/or their cleavage products). This isolation procedure is simpler and cheaper than isolation of DNA with popular DNA Lego Kit, which we introduced to the market more than a decade ago. DNA isolated using Column DNA Lego Kit can be used for variety of molecular-biology techniques such as sequencing, cloning, PCR, restriction enzymes cutting, mutagenesis *in vitro*, transcription *in vitro,* labeling reactions, transformation, or transfection. Advantage of the Column DNA Lego Kit is that its components can be bought separately and universally used for isolation of (1) plasmid DNA, (2) DNA fragments from agarose gels, (3) DNA from solutions, including amplified DNA after PCR, and (4) genomic DNA.

Technical data

Unit definition

- Column DNA Lego Kit allows 200 isolations of pure DNA samples.

Storage

- At room temperature (+15 to +25^{o}C), except RNase which should be stored at -15 to -25^{o}C freezer and Suspension buffer [P1] with RNase stored at fridge (+2 to +8^{o}C) where it will be stable for 6 months. Storage of the Kit at fridge may lead to formation of salt precipitates in the buffers, which should disappear at room temperature. The Kit is shipped at ambient temperature.

Components

Column DNA Lego Kit (200 purifications):

- DNA bind columns (200 pcs) and collection tubes (200 pcs) in 4 boxes
- DNA bind buffer [L1], 60 ml
- Wash buffer [L2], 250 ml
- Elution buffer [L3], 20 ml
- Instructions

Column DNA Lego Kit + plasmid supplement (200 purifications), possesses all components of the Column DNA Lego Kit and furthermore 4 solutions required for plasmid DNA isolation

- Suspension buffer [P1], 50 ml
- RNase A, DNase-free (10 mg/ml), 0.5 ml
- Lysis buffer - blue [P2], 50 ml
- Neutralization buffer [P3], 10 ml

Protocols

For DNA isolation with Column DNA Lego Kit the following laboratory devices are required: laboratory tabletop microcentrifuge capable of 13.000 x g centrifugal force (e.g. Eppendorf 5415C or equivalent), pipetting devices, tips, vacuum pump, refrigerator, freezer, water bath or heating devices, and centrifugation tubes Eppendorf.

1. Isolation of plasmid DNA from bacterial culture (miniprep)

The method is based on alkaline lysis protocol, followed by neutralization and binding of the plasmid to silica membranes in the presence of chaotropic agents (guanidine thiocyanate; GITC). After washing out of unbound material, pure DNA is released from the membranes into elution buffer during centrifugation. Overview of the plasmid DNA isolation is shown in Figure 1.

1.1. Bacteria are produced in overnight cultures (density of 1.5 - 5.0 A_{600}) in a volume of more than 1.5 ml in the presence of proper antibiotic.

1.2. Suspension of bacteria cells is transferred into 1.5 ml Eppendorf tube and the cells are sedimented by centrifugation at 6.000 x g for 30 s.

1.3. Supernatant is carefully removed and bacterial pellet is resuspended by pipetting or vortexing with 250 µl of the **Suspension buffer** + **RNase [P1].**

1.4. **Lysis buffer - blue [P2]** (250 µl) is then added and the sample is slowly mixed (inverting it 3 to 6 times; no vortexing), followed by incubation for 5 min at room temperature (between +15 to +25^{o}C; longer exposure could denature the plasmids).

1.5. **Neutralization buffer [P3]** (50 µl) and **DNA bind buffer [L1]** (300 µl) are then added. Samples are gently mixed by repeated inverting until blue color disappears. The samples are left on ice for 5 min (the solution should become cloudy) and then centrifuged at 13,000 x g for 10 min.

1.6. Supernatant (800 µl) is then transferred to the column on collection tube and the set is inserted into rotor of the tabletop microcentrifuge. The samples are centrifuged at 13,000 x g for 1 min.

1.7. After centrifugation the flowthrough liquid is discarded and the column is inserted into the same collection tube. Then, **Wash buffer [L2**] (700 µl) is added to the column set and the sample is centrifuged at full speed for 1 min.

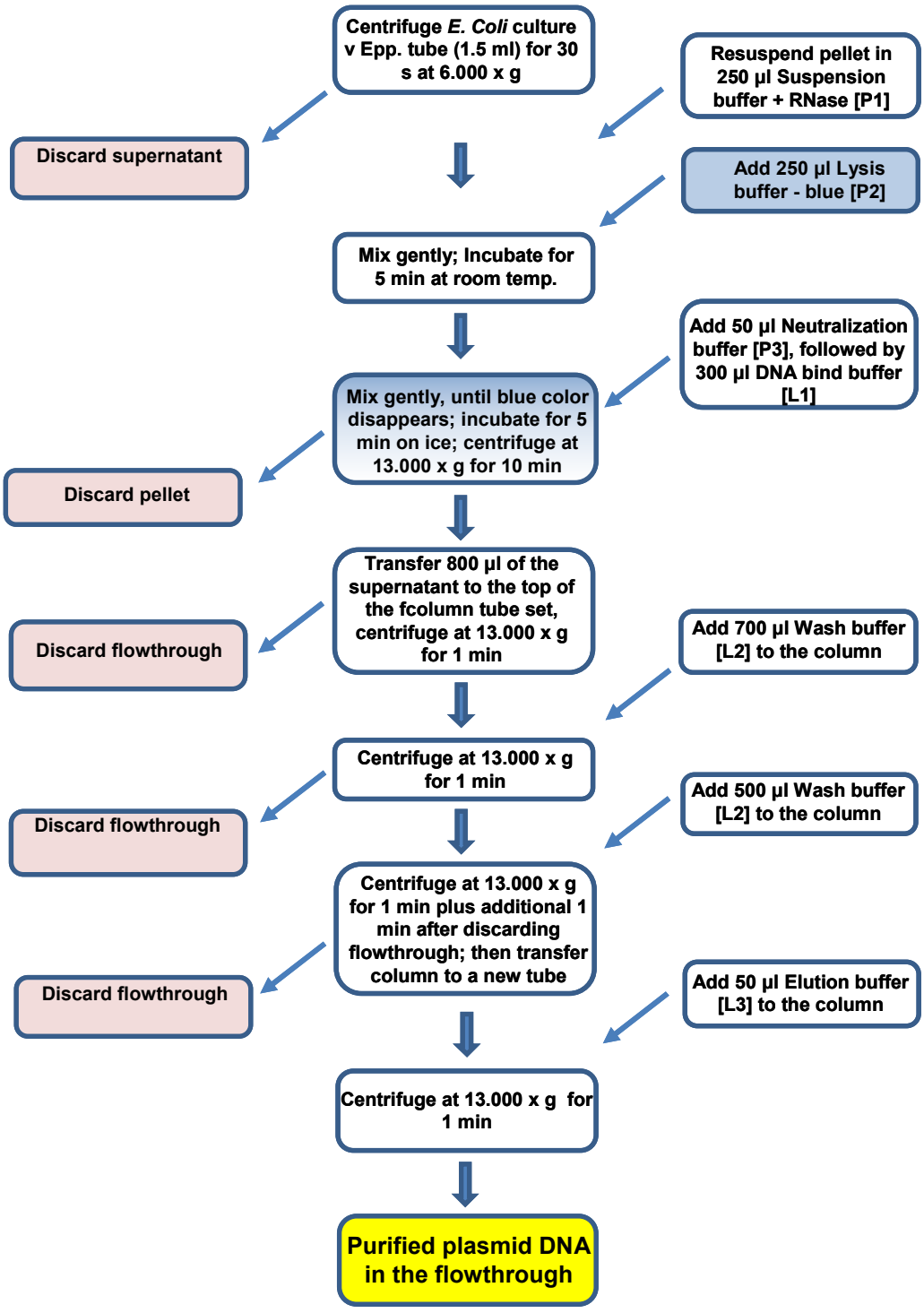
1.8. Flowthrough in the collection tube is discarded and **Wash buffer [L2**] (500 µl) is added to the column. The samples are centrifuged at 13,000 x g for 1 min.

1.9. Flowthrough in the collection tube is again discarded and the column on the collection tube is centrifuged once more at 13,000 x g for 1 min, to remove all residual wash buffer.

1.10. Column with bound plasmid is inserted into a clean 1.5 ml Eppendorf collection tube and 50 µl of **Elution buffer** [L3] is added. After 2 min the tube is centrifuged at full speed for 1 min. For more efficient elution of DNA, another 50 µl of **Elution buffer** may be applied to the column and centrifugation repeated. Elution buffer can be heated to 50^{o}C to increase the plasmid yield.

1.11. The collection tube contains the eluted plasmid DNA, which can be used directly for cloning, sequencing or other applications or stored at +2 to +8^{o}C or -15 to -80^{o}C for later analysis.

Figure 1. Overview of plasmid DNA isolation (miniprep)



2. Isolation of DNA fragments from agarose gels

This method is based on solubilization of agarose gel in DNA bind buffer (containing GITC) and binding of DNA to DNA bind columns in the presence of GITC. After washing out of unbound material, pure DNA is released by elution buffer during centrifugation.

2.1. From agarose gel a piece containing DNA is cut out using a scalpel or blade. The gel is weight and for each gram of the gel 3 ml of **DNA bind buffer [L1]** is added. Size of the gel piece should be minimal as some agarose batches possess inhibitors interfering with the binding of DNA to DNA bind columns. Better recovery of DNA is obtained when low melting agarose is used.

2.2. Agarose gel is dissolved by incubation the sample for 5 - 10 min at 55^{o}C.

2.3. Solution containing dissolved agarose gel and DNA (< 800 µl) is transferred to the column on collection tube. The tube assembly is transferred into microcentrifuge rotor and the sample is centrifuged at 13,000 x g for 1 min. The process is repeated if more solution is produced.

2.4. After centrifugation the flowthrough liquid is discarded and the column is reinserted in the same collection tube. Then, **Wash buffer [L2**] (700 µl) is added to the column, followed by centrifugation at 13,000 x g for 1 min

2.5. Flowthrough in the collection tube is discarded and again **Wash buffer [L2**] (500 µl) is added to the column, followed by centrifugation at 13,000 x g for 1 min.

2.6. Flowthrough in the collection tube is again discarded and the column is centrifuged once more at 13,000 x g for 1 min, to remove all residual wash buffer.

2.7. Column with bound DNA is inserted into a clean, sterile 1.5 ml Eppendorf collection tube and **Elution buffer** [L3] (50 µl) is added to the column. After 2 min the column set is centrifuged at 13,000 x g for 1 min. For more efficient elution of DNA, another aliquot of the Elution buffer can be applied to the column and centrifugation repeated. Further increase of DNA elution can be obtained by heating Elution buffer to 50^{o}C before adding to the column.

2.8 Pure DNA collected in the Eppendorf tube can be used directly for further manipulation or stored at +2 to +8^{o}C or -15 to -80^{o}C for later use.

3. Isolation of DNA from solutions

This method is based on mixing DNA-containing samples with DNA bind buffer, followed by binding of DNA to DNA bind column. After washing out of the unbound material, pure DNA is released by elution buffer during centrifugation.

3.1. Liquid sample containing DNA (e.g. DNA amplified by PCR) is mixed with two-fold volume of **DNA bind buffer [L1]** and incubated at least 1 min at room temperature.

3.2. The sample (<800 µl) is transferred to the column on collection tube. The column assembly is transferred into a microcentrifuge and centrifuged at 13,000 x g for 1 min. The process is repeated if more solution is produced at step 3.1.

3.3. After centrifugation the flowthrough liquid is discarded and the column is reinserted in the same collection tube. Then, **Wash Buffer [L2]** (700 µl) is added to the column, followed by centrifugation at 13,000 x g for 1 min.

3.4. Flowthrough in the collection tube is discarded and Wash buffer [L2] (500 µl) is added to the column, followed by centrifugation at 13,000 x g for 1 min.

3.5. Flowthrough in the collection tube is again discarded and the column on collection tube is centrifuged once more at 13,000 x g for 1 min, to remove all residual wash buffer.

3.6. Column with bound DNA is inserted into a clean 1.5 ml Eppendorf collection tube and 50 µl **Elution buffer [L3]** is added. After 2 min the tube is centrifuged at full speed for 1 min. For more efficient elution of DNA, another 50 µl

aliquot of elution buffer may be applied to the column and centrifugation is repeated. Elution buffer may be heated to 50°C to increase the plasmid yield.

3.7. The collection tube now contains the eluted DNA, which can be used directly for further analysis or stored at +2 to +8°C or -15 to -80°C for later analysis.

4. Isolation of genomic DNA

Genome DNA isolated by this procedure is suitable for PCR and qPCR amplifications

4.1. Cell suspension is prepared in PBS. Alternatively, human or animal blood is collected into EDTA [e.g. by mixing 50 µl of 50 mM EDTA with 500 µl of blood].

4.2. In 1.5 ml Eppendorf tube 3 parts of **DNA bind buffer [L1] is mixed with** 1 part (or less) of cell suspension or blood in EDTA.

4.3. The mixture is incubated for 10 min at room temperature with gentle mixing every 2 min.

4.4. The tube is spun down on microfuge at 6000 x g for 30 s.

4.5. Supernatant (<800 µl) is transferred to the column on collection tube. The column/tube assembly is transferred into a rotor of a microcentrifuge and centrifuged at 13,000 x g for 1 min. The process is repeated if more solution is produced in step 4.2.

4.6. The flowthrough liquid is discarded and the column is reinserted in the same collection tube. Then, **Wash Buffer [L2]** (700 µl) is added to the column, followed by centrifugation at 13,000 x g for 1 min.

4.7. Flowthrough in the collection tube is discarded and **Wash buffer [L2]** (500 µl) is added to the column, followed by centrifugation at 13,000 x g for 1 min.

4.8. Flowthrough in the collection tube is again discarded and the column is centrifuged once more at 13,000 x g for 1 min, to remove the residual wash buffer.

4.9. The column with bound DNA is inserted into a clean 1.5 ml Eppendorf collection tube and 50 µl **Elution buffer [L3]** is added. After 2 min the tube is centrifuged at 13,000 x g for 1 min. For more efficient elution of DNA, another 50 µl elution buffer can be applied to the column and centrifugation is repeated. Elution buffer can be heated to 50°C to increase the plasmid yield.

4.10. The collection tube now contains the eluted DNA, which can be used directly for further analysis or stored at +2 to +8°C or -15 to -80°C for later analysis.

Cat. No.	Product name and specification	Quantity
D201	**Column DNA Lego Kit,** 200 isolations	**1 kit**
D202	**Column DNA Lego Kit + plasmid supplement,** 200 reactions	**1 kit**
Spare parts		
D203	DNA bind columns (50) and collection tubes (50)	1 box
D204	DNA bind buffer [L1]	60 ml
D205	Wash buffer [L2]	250 ml
D206	Elution buffer [L3]	20 ml
D207	Suspension buffer [P1]	50 ml
D106	RNase A, DNase-free (10 mg/ml)	0,5 ml
D208	Lysis buffer - blue [P2]	50 ml
D209	Neutralization buffer [P3]	10 ml