PRODUCT INFORMATION

Product Name : Gel Indicator™ DNA Extraction Kit
Code No. : DM550 *This product is research use only*

Kit Components: 50 reactions

Code #	Components	Contents	Size
DM520	3-Colors Indicator	Xylen cyanol FF, Bromophenol blue, Orange G, Tris-HCl buffer, Sodium EDTA , Glycerin < DNase free >	1 ml
DM530	6×Orange Loading Dye	Orange G, Tris-HCl buffer, Sodium EDTA, Glycerin < DNase free >	1 ml
DM540	Gel Indicator Solution	Dye < DNase free >	1 ml × 3
DM561	Dissolving & Binding Buffer	Sodium Acetate, Sodium Perchlorate, Mannitol	50 ml
DM562	Wash Buffer	Sodium Chloride, EDTA Sodium Salt, Tris –HCl buffer	15 ml
	Add 35 ml of 100 % ethanol to the Wash Buffer before use.		
DM563	Elution Buffer	10 mM Tris-HCl buffer	10 ml
DM571	Spin Column		50
DM572	Collection Tube		50

Introduction: Gel Indicator™ DNA Extraction Kit is manufactured for DNA purification from agarose gel without DNA damege of UV light. The Purification of DNA from agarose gel is often used for DNA-cloning work. However conventional method of visualizing DNA with ethidium bromide and exposure of UV light damages DNA and significantly decreases cloning efficiency. Using Gel Indicator™ DNA Extraction Kit, UV light exposure is not required, and DNA can be excised under visible light. Because of no UV damage, obtained DNA shows higher cloning efficiency than one using ethidium bromide and UV light. Although the sensitivity of DNA detection with Gel Indicator Solution is less than by the method of ethidium bromide and UV light exposure, DNA is detectable as much as 50 ng by DNA excision, protocol II. Purified DNA with Gel Indicator™ DNA Extraction Kit is high-quality and the DNA is suitable for many applications, such as enzyme digestion, sequencing, PCR, *in vitro* transcription, as well as cloning.

Feature and Specification:

- Excision of DNAs on your bench. Without ethidium bromide, DNA bands are visible.
- No damaged DNA by UV is purified.
- Extraction is possible from agarose gel electrophoresis with 1×TAE or 0.5×TBE.
- Purified DNA is suitable for many applications, cloning, enzyme digestion, sequencing, PCR.
- Recommended DNA size for excision: 250 (100*) - 10, 000 bp
- DNA detection limit / band on agarose gel: 500 ng (50 ng*) * DNA excision, protocol II
- Capacity of DNA binding of one Spin Column is up to 8 μg.
- DNA (linear) recovery from one Spin Column is 80-90 %.
- Linear DNA is obtained more preferentially than circular DNA.
  (The yield of circular DNA is less than 10 %.)

Storage condition : Stable at 15 to 25°C for six months from the date of receipt.

Ver. 1.5

BioDynamics Laboratory Inc. 9-7 HONGO 2-CHOME,BUNKYO-KU,TOKYO113-0033,JAPAN
http://www.biodynamics.co.jp

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Additional Materials Needed :

Following materials are required.

- Agarose for DNA electrophoresis
- 1×TAE buffer or 0.5×TBE buffer
- Microwave
- Electrophoresis apparatus
- Tray for staining agarose gel (DNA excision, protocol II)
- Razor blade
- Ethanol
- Microcentrifuge
- 1.5 ml of centrifuge tubes
- Heat block for 50-55 ^{o}C incubation

Caution !

Wear gloves and protecting clothing while handling this kit. The stain of the Gel Indicator Solution is strong.

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Protocol of DNA excision and purification:

It can carry out from DNA excision to purification, using Gel Indicator™ DNA Extraction Kit. There are two protocols for DNA excision process. One is a rapid method, DNA can be excised just after electrophoresis. Another is high-sensitive DNA detection procedure. DNA is detectable as much as 50 ng.

1. DNA excision

1.1. DNA excision, protocol I

Feature and information

- DNA can be excised just after electrophoresis.
- The 3-color indicator let know approximate sizes of DNA
- Recommended DNA size for excision: 250 - 10, 000 bp
- DNA detection limit / band on agarose gel: 500 ng

1) Preparation of gel

To 100 ml of 1×TAE* buffer, add 0.8 g of powdered agarose for DNA separation. Mix them and dissolve agarose in the microwave. Add 100 µl of Gel Indicator Solution to the agarose (100 ml) after removing from microwave. Mix them by swirling and pour the agarose into mold and set a comb. Allow the gel to harden.

2) Sample preparation

Dye in the Gel Indicator Solution has a lower sensitivity than ethidium bromide. DNA sample, restriction digest or PCR product, should be loaded greater than 500 ng/lane. Mix 10 µl of DNA Sample (>500 ng) and 2 µl of 6×Orange loading Dye and load to a well. Load 10 µl of 3-Colors Indicator to a well for a reference indicator. Do not mix the 3-Colors Indicator and DNA samples. The 3-Colors Indicator contains xylen cyanol FF, bromophenol blue and orange G. They migrates at approximately the same rate as 6,000, 700, 50 bp of DNAs in 0.8 % agarose gel in 1×TAE, although the relationships are affected by run time of electrophoresis.

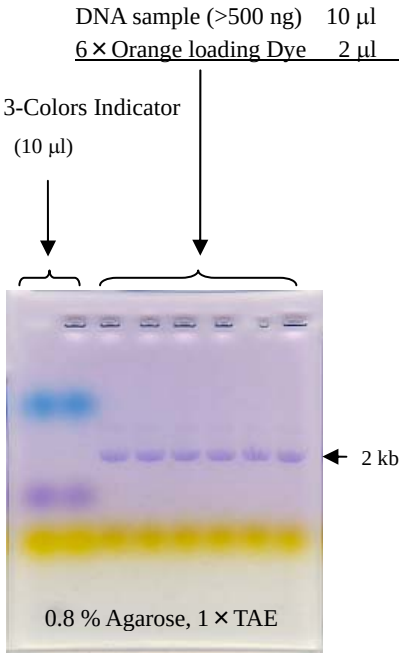

Fig 1. Electrophoresis by protocol I
DNA sample, 2 k bp, is mixed with 6×Orange Loading Dye. Then load to wells according to the protocol I.

3) Excising DNA

During electrophoresis DNA can be visible as a blue band. DNA can be excised from agarose gel under normal light with a razor blade.

* Instead of 1×TAE, 0.5×TBE also can be used.

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1.2. DNA excision, protocol II

Feature and information

- After electrophoresis, agarose gel is stained with diluted Gel Indicator Solution.
- DNA detection limit / band on agarose gel: 50 ng
- Recommended DNA size for excision: 100 - 10, 000 bp

1) Agarose gel and electrophoresis

Prepare 0.5-2.0 % agarose gel with 1×TAE* buffer. Gel concentration depends on DNA size to be separated. Set the agarose gel into an electrophoresis apparatus with 1×TAE* buffer. Mix 10 µl of DNA Sample (>50 ng) and 2 µl of 6×Orange loading Dye. Load the mixed sample to a well and start electrophoresis.

2) Preparation of diluted Gel Indicator Solution (0.1 %).

Mix well one volume of Gel Indicator Solution and 1000 volume of pure water or 1 × TAE* (running buffer used for electrophoresis). For example, 100 µl of Gel Indicator Solution should be mixed with 100 ml of pure water. Prepare enough 0.1 % Gel Indicator Solution to immerse the agarose gel. Transfer the 0.1 % Gel Indicator Solution to a tray for staining.

3) Staining with 0.1 % Gel Indicator Solution

After electrophoresis, the agarose gel is transferred to a tray containing the 0.1 % Gel Indicator Solution. Stain the agarose gel over 2 hr with gentle agitation. When DNA is visible as a blue band, DNA can be excised from agarose gel with a razor blade or gel-excision devices, for example, x-tracta (LabGadget, USA, Funakoshi, Japan).

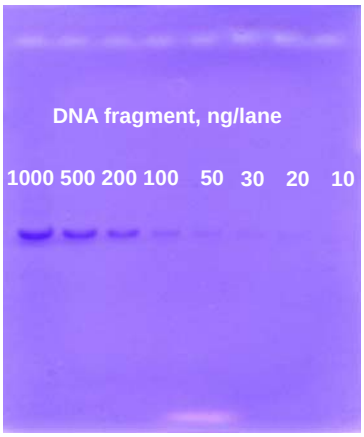

Fig. 2. Detection of DNA by Protocol II. Serially diluted DNA fragment (2 k bp) was loaded to wells from right to left lanes. After electrophoresis, the agarose gel was stained with diluted Gel Indicator Solution.

Agarose Electrophoresis (0.8 %), 1×TAE

* Instead of 1×TAE, 0.5×TBE also can be used.

‡ Reuse of diluted Gel Indicator Solution is not recommended.

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2. DNA extraction and purification

Before start:
• Add 35 ml of 100 % ethanol to 15 ml of Wash Buffer.
• Prepared 50-55 °C heat block, or bath.

1) Transfer the gel slice to a 1.5 ml microcentrifuge tube and add two volume (or 2.5-3.0 volumes, if agarose gel concentration is over 1.5 %) of Dissolving & Binding Buffer.
* The maximum amount of gel per one Spin Column is 400 mg and maximum volume of one column reservoir is 800 µl. If volume of dissolved gel is over 800 µl, load less than 800 µl and spin, then load remained one. Do not load over 400 mg of gel per one Spin Column.

2) Incubate the microcentrifuge tube containing the gel at 50-55 °C for 10-15 min.

3) Insert a Spin Column into a Collection Tube. After the gel slice is completely dissolved, transfer the dissolved gel to a Spin Column and centrifuge for 1 min at 7,000-10,000×g.

4) Discard the filtrate in the Collection Tube.

5) (Optional. Add 500 µl of Dissolving & Binding Solution and centrifuge for 1 min at 7,000-10,000×g. Discard the filtrate in the Collection Tube. The optional procedure removes residual agarose, which is recommended to obtain DNA of higher purity but an yield of DNA may slightly decrease.)

6) Add 700 µl of Wash Buffer (contains EtOH) and centrifuge for 1 min at 7,000 -10,000×g. Discard the filtrate in the Collection Tube.

7) Centrifuge the Spin Column again for 1 min at 7,000-10,000×g. to remove residual Wash Buffer.

8) Transfer the Spin Column into a new microcentrifuge tube. Add 50 µl of prewarmed Elution Buffer (50-55 °C) onto the Spin Column and incubate it at room temperature for 1 min, then centrifuge the Spin Column to elute DNA, for 1 min at 7,000-10,000×g. Thus eluted DNA is ready for many applications.