Strong DNA Gel Mark Kit

Catalog No. DGM02

Kit Components:

No.	Components	Contents	Cap	Amount
1	3-Colors Marker (Do not mix with DNA samples)	Bromophenol blue, Xylene cyanol FF, Orange G, Tris-HCl buffer, EDTA, Glycerol	White	0.5 ml
2	6 X Loading Dye	Orange G, Tris-HCl buffer, EDTA, Glycerol	Orange	0.5 ml x 3
3	DNA Gel Mark Solution	Dye	Purple	1.5 ml x 2

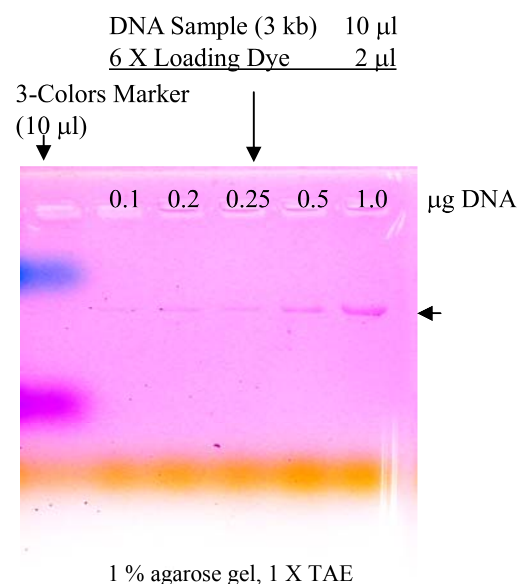
Introduction:

Recovering DNA from agarose gels is used in DNA-cloning experiments routinely. However, the conventional method of visualizing DNA with ethidium bromide and UV light exposure damages DNA and significantly decreases cloning efficiency (1, 2). The DNA Gel Mark solution in this kit does not contain ethidium bromide as DNA-staining dye. Although the sensitivity of DNA detection by DNA Gel Mark Solution is less than the method of ethidium bromide and UV light exposure. DNA is still detectable while the amount is 100 ng. By using this kit, DNA can be excised under visible light and get higher cloning efficiency than detection by ethidium bromide and UV light. All components in this kit are tested DNase free. The excised DNA from agarose gel can be further purified by organic solvent extraction or other commercial kits.

Storage: Kit is stable until expiry date (see lot-specific label imprint) at 2-8°C.

Protocol I :

- ***DNA bands are visible during electrophoresis.***
- ***DNA can be excised intermediately after electrophoresis.***
- ***The 3-colors Marker let you know approximate sizes of DNA***
- ***Recommended DNA size for recover: 200 - 10, 000 bp***
- ***DNA detection limit per band on agarose gel: 100 ng***



1 % agarose gel, 1 X TAE

1. Preparation of gel
   To 50 ml of 1 X TAE buffer, add 0.5 g of powdered agarose for DNA separation. Mix well and dissolve agarose in the microwave. Add 50 µl of Gel Mark Solution to the agarose (50 ml) after removing from microwave. Mix by stirring and pour the agarose into gel cast and set a comb. Allow the gel to harden.
2. Sample preparation
   Dye in the Gel Mark Solution has a lower sensitivity than ethidium bromide. DNA sample to be excised, restriction digest or PCR product, should be greater than 100 ng. Mix 10 µl of DNA Sample (>100 ng /band) and 2 µl of 6 X Loading Dye and load into a well. Load 10 µl of 3-Colors Marker into a well for a reference. Do not mix the 3-Colors Marker and DNA samples. The 3-Colors Marker contains xylene cyanol FF, bromophenol blue and orange G. They migrates at approximately the same size as 6,000, 700, 50 bp of DNAs in 1.0 % agarose gel with 1 X TAE, though the relationships are affected by gel percentage of electrophoresis.
3. Excising DNA band
   During electrophoresis, DNA fragments are visible as blue bands. DNA can be excised from agarose gel under visible light with a razor blade. The excised DNA with this kit can be purified by organic solvents extraction or with other commercial kits.

Protocol II :

- ***After electrophoresis, agarose gel is stained with diluted Gel Mark Solution.***
- ***Recommended DNA size for excision: 100 - 10, 000 bp***
- ***DNA detection limit per band on agarose gel: 20 ng***

1. Agarose gel and electrophoresis
   Prepare 0.5-2.0 % agarose gel with 1 X TAE buffer. Gel concentration depends on DNA size to be separated. Set the agarose gel into an electrophoresis apparatus with 1 X TAE buffer. Mix 10 μl of DNA Sample (>50 ng) and 2 μl of 6 X loading Dye. Load the mixed sample to a well and start electrophoresis.
2. Preparation of 0.1 % diluted Gel Mark Solution
   Make 1000 fold dilution for DNA Gel Mark Solution. Mix one volume of Gel Mark Solution with 1000 volume of pure water or 1 X TAE (used after electrophoresis). For example, mix 100 μl of Gel Mark Solution with 100 ml of pure water.
3. Staining with 0.1 % Gel Mark Solution
   After electrophoresis, the agarose gel is transferred to a tray containing 0.1 % Gel Mark Solution. Make sure that the agarose gel is immersed in diluted Gel Mark Solution completely. Stain the agarose gel 1-2 hr with gentle agitation. When DNA is visible as a blue band, excise needed band from agarose gel with a razor blade for DNA recovery.

§ Reuse of diluted Gel Mark Solution is Not recommended.

Additional Materials Needed

To excise DNA bands from agarose gel, the following materials are required.

- Agarose for DNA electrophoresis
- 1 X TAE buffer
- Microwave
- Electrophoresis apparatus
- Razor blade

Note: Wear gloves and protecting clothing while handling this kit. The stain of the DNA gel mark solution is strong .

Reference:

1. Cariello NF, Keohavong P, Sanderson BJ, Thilly WG., (1988) DNA damage produced by ethidium bromide staining and exposure to ultraviolet light. Nuc. Acids. Res., **16**, 4157.
2. Hartman P.S., (1991) Transillumination can profoundly reduce transformation frequencies. Biotechniques, **11**, 747-748.