Thermo SCIENTIFIC

PRODUCT INFORMATION

# Silica Bead DNA Gel Extraction Kit

# #K0513

www.thermoscientific.com/onebio

#K__
Lot ___

CERTIFICATE OF ANALYSIS

This lot of the kit has been tested in the purification of 1 µg of a 950 bp and 120 bp DNA fragment from a 1% agarose gel. The recovery of DNA fragments was evaluated electrophoretically in agarose gel.

Quality authorized by:  Jurgita Zilinskiene

CONTENTS page

## COMPONENTS OF THE KIT

| Silica Bead DNA Gel Extraction Kit | 100 preps<br>of up to 5 µg of DNA<br>#K0513 |
|---|---|
| Silica Powder Suspension<br>(glass bead suspension in water) | 1.5 ml |
| Binding Buffer | 125 ml |
| Concentrated Washing Buffer | 15 ml |
| TBE Conversion Buffer | 15 ml |

## STORAGE

The Silica Bead DNA Gel Extraction Kit should be stored at 4°C before use. The diluted wash buffer should be stored at -20°C.
If used infrequently, the silica powder suspension should be aliquoted and tubes which are not in use should be stored at -20°C.

## DESCRIPTION

The Silica Bead DNA Gel Extraction Kit is designed for efficient extraction of DNA from agarose gels and reaction mixtures. It provides a rapid method to separate DNA fragment of interest from primers, unincorporated nucleotides, excess linkers, enzymes and salts, residual phenol, chloroform or dyes (ethidium bromide, bromphenol blue, etc.).
The Silica Bead DNA Gel Extraction Kit can be used to purify a wide range of DNA fragments with recoveries of >80%. DNA as small as 120 bp can be purified with this kit.
The entire procedure takes approximatelly 20 minutes and the isolated DNA can be used directly in common downstream molecular biology applications including restriction digestion, cloning, sequencing, etc.

## PRINCIPLE

The Silica Bead DNA Gel Extraction Kit utilizes the modified glass beads protocol of Vogelstein and Gillespie (1). In the presence of chaotropic salts DNA binds to the specially prepared glass particles. As an added convenience, the binding buffer contains a color indicator that allows for easy monitoring of the solution pH for optimal DNA binding. The chaotropic salts and impurities are washed from the glass particles containing bound DNA. The washing steps are followed by elution of the DNA in TE buffer or water.

## IMPORTANT NOTES

- Prior to the initial use, dilute the Concentrated Washing Buffer:

| | |
|---|---|
| Concentrated Washing Buffer | 15 ml |
| Distilled water | 285 ml |
| Ethanol (95-100%) | 300 ml |
| Total Volume | 600 ml |

- TBE can inhibit the ability of DNA to bind to the glass beads. The TBE Conversion Buffer included in the kit neutralizes the inhibitory effects of TBE buffer and therefore should be used during purification of DNA fragments from TBE agarose gels.
- The Silica Powder Suspension is not damaged if it is dried. If the suspension becomes dry during prolonged storage, add sterile distilled/deionized water to the tube so that the amounts of liquid and solid are approximately equal. The addition of the water will completely reconstitute the Silica Powder Suspension.
- If the kit is used infrequently or if the kit has several users, aliquot the Silica Powder Suspension into several tubes. Store the tubes not in use at -20°C.

## ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Ethanol 96-100%.
- 3 M sodium acetate, pH 5.2 (may be necessary).
- TE (Tris-EDTA) buffer.
- Microcentrifuge.
- 1.5 or 2 ml microcentrifuge tubes.
- Heating block or water bath.

Note

- Read IMPORTANT NOTES on p. 3 before starting.
- All purification steps should be carried out at **room temperature**.
- All centrifugations should be carried out in a table-top microcentrifuge at >12000 x g (10 000-14 000 rpm, depending on the rotor type).

## A. DNA purification from agarose gel

| Step | Procedure |
|---|---|
| 1 | Excise gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 ml tube and weigh. Record the weight of the gel slice.<br>Note<br>If the purified DNA will be used for cloning, avoid UV damage of the DNA by minimizing the UV exposure to a few seconds or keeping the gel slice on a glass or plastic plate during UV illumination. |
| 2 | Add a **3:1 volume** of **Binding Buffer** to the gel slice (volume:weight) (e.g., add 300 µl of Binding Buffer for every 100 mg of agarose gel).<br>Incubate the gel mixture at 55°C for 5 min or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process.<br>Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.<br>Note<br>If the DNA is extracted from a TBE agarose gel, add ½ volume of TBE Conversion Buffer and 4.5 volumes of Binding Buffer to a given volume of agarose. |
| 3 | Add the resuspended **Silica Powder Suspension** to the DNA/Binding Buffer mixture.<br>For ≤2.5 µg of DNA add 5 µl of Silica Powder Suspension.<br>For >2.5 µg of DNA add 2 µl of Silica Powder Suspension per µg of DNA.<br>Incubate the mixture for 5 min at 55°C to allow for binding of the DNA to the silica matrix. Mix by vortexing every few minutes to keep the silica powder in suspension.<br>Note<br>If a large amount of DNA is purified or if the volume of the binding reaction is greater than 1.5 ml increase the incubation time of the binding step to 15 min. |
| 4 | Spin the silica powder/DNA mixture for 5 s to form a pellet. Carefully remove the supernatant solution and discard.<br>*(Optional)*. The pelleted silica powder/DNA complex can be resuspended with an additional 300 µl of Binding Buffer to dissolve any residual undissolved agarose from step 2. Place the suspension in a 55°C water bath for a few minutes and proceed to step 5. |

| Step | Procedure |
|---|---|
| 5 | Add **500 µl** of ice cold Washing Buffer (diluted with ethanol as described on p. 3), resuspend the pellet and spin for 5 s. Discard the supernatant. Repeat this procedure three times.<br><br>After the supernatant from the last wash has been removed, spin the tube again and remove the remaining liquid with a pipette.<br><br>If necessary air-dry the pellet for 10-15 min to avoid the presence residual ethanol in the purified DNA solution. Residual of ethanol in the DNA sample may inhibit downstream enzymatic reactions.<br><br>Note<br>• To obtain efficient washing, the pellet should be resuspended completely. Resuspend the pellet in the Washing Buffer by vortexing, pipetting the solution back and forth onto the pellet or manually resuspending the pellet with a pipette tip.<br>• For DNA fragments ≥5 kb, resuspend the pellet by inverting the tube. Vortexing can cause shearing of large DNA molecules |
| 6 | Resuspend the pellet in the desired volume of sterile deionized water or TE and incubate the tube at 55°C for 5 min.<br><br>Spin the tube and remove the supernatant while avoiding the pellet. Place the recovered supernatant into a fresh tube and repeat the elution with another aliquot of water or TE.<br><br>For the removal of residual silica powder, spin the tube again for 30 s in a table-top centrifuge and transfer the supernatant into a new tube.<br><br>Note. The optimal elution volume is equal to the volume of the silica powder suspension that was added in step 3. |

## B. DNA purification from reaction mixture

| Step | Procedure |
|---|---|
| 1 | Add a 3:1 volume of Binding Buffer to the reaction mixture (e.g., for every 100 µl of reaction mixture, add 300 µl of Binding Buffer). Mix thoroughly.<br>Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow. |
| 2 | Add the resuspended Silica Powder Suspension to the DNA/Binding Buffer mixture.<br>For ≤2.5 µg of DNA add 5 µl of Silica Powder Suspension.<br>For >2.5 µg of DNA add 2 µl of Silica Powder Suspension per µg of DNA.<br>Incubate the mixture for 5 min at 55°C to allow for binding of the DNA to the silica matrix. Mix by vortexing every few minutes to keep the silica powder in suspension.<br>Note<br>If a large amount of DNA is purified or if the volume of the binding reaction is greater than 1.5 ml increase the incubation time of the binding step to 15 min. |
| 3 | Spin the silica powder/DNA complexes for 5 s to form a pellet. Carefully remove the supernatant solution and discard. |
| 4 | Add **500 µl** of ice cold Washing Buffer (diluted with ethanol as described on p. 3), resuspend the pellet and spin for 5 s. Discard the supernatant. Repeat this procedure three times.<br>After the supernatant from the last wash has been removed, spin the tube again and remove the remaining liquid with a pipette.<br>If necessary air-dry the pellet for 10-15 min to avoid the presence of residual ethanol in the purified DNA solution. Residual ethanol in the DNA sample may inhibit downstream enzymatic reactions.<br>Note<br>• To obtain efficient washing, the pellet should be resuspended completely. Resuspend the pellet in the Washing Buffer by vortexing, pipetting the solution back and forth onto the pellet or manually resuspending the pellet with a pipette tip.<br>• For DNA fragments ≥5 kb, resuspend the pellet by inverting the tube. Vortexing can cause shearing of large DNA molecules |
| 5 | Resuspend the pellet in the desired volume of sterile deionized water or TE and incubate the tube at 55°C for 5 min.<br>Spin the tube and remove the supernatant while avoiding the pellet. Place the reovered supernatant into a fresh tube and repeat the elution with another aliquot of water or TE.<br>For the removal of residual silica powder spin the tube again for 30 s in a table-top centrifuge and transfer the supernatant into a new tube.<br>Note. The optimal elution volume is equal to the volume of the silica powder suspension that was added in step 2. |

TROUBLESHOOTING

| Problem | Possible Cause and Solution |
|---|---|
| Low DNA yield | Incomplete solubilization of the gel slice.<br>Use a 3:1 volume of Binding Buffer for a precisely weighted gel slice (e.g., for every 100 mg of agarose gel, add 300 µl of Binding Buffer). Ensure that the gel slice is completely dissolved before adding Silica Powder Suspension.<br>A large amount of agarose or a gel slice with an agarose percentage greater than 2% may require extra time to dissolve.<br>Additional vortexing of the gel solution facilitates solubilization.<br><br>Inefficient DNA binding.<br>Use the correct amount of Silica Powder Suspension for certain amount of DNA (for up to 2.5 µg of DNA add 5 µl of Silica Powder Suspension, above 2.5 µg of DNA add 2 µl of Silica Powder Suspension per each 1 µg of DNA).<br>If a large amount of DNA is purified or if the volume of binding reaction is greater than 1.5 ml increase the incubation time of binding step for an additional 15 min. Overnight incubations are not recommended.<br>If the DNA is extracted from a TBE agarose gel, add ½ volume of TBE Conversion Buffer and 4.5 volumes of Binding Buffer to a given volume of agarose.<br>If the DNA is not bound efficiently to the Silica Powder Suspension, even when isolating DNA from a gel or solution which does not contain TBE buffer, add a 1/10 volume of TBE Conversion Buffer to Binding Buffer/DNA mixture. This will increase the binding of the DNA to silica matrix.<br><br>Incorrect washing.<br>Ensure that the recommended volume of ethanol (95-100%) was added to the Concentrated Washing Buffer prior to the first use (*see* p. 3). The volume of the ethanol is important because the ability of the Washing Buffer to keep DNA bound to the silica powder diminishes if the final concentration of ethanol is less than 50%.<br>Do not dry the pellet under vacuum, air-dry for 10-15 min if necessary.<br><br>Inefficient DNA elution.<br>Insufficient volume of water or TE buffer was used for elution. Repeat the elution step 2-3 times with new aliquots of TE or water. |

| Problem | Possible Cause and Solution |
|---|---|
| Downstream applications are unsuccessful | Residual ethanol in the eluate.<br>After washing ensure that all residual wash buffer is removed from the pellet. After the supernatant from the last wash has been removed, spin the tube again and remove the remaining liquid with a pipette. Air-dry the pellet for 10-15 min.<br>Presence of residual silica beads in the eluate.<br>Ensure the pellet is not disturbed during recovery of the supernatant after elution. If necessary, centrifuge DNA solution again and transfer the supernatant to a new microcentrifuge tube.<br>Excess salt in the eluate.<br>Ensure that the wash step is effective. During each wash cycle the pellet should be resuspended completely by vortexing, pipetting back and forth over the pellet or manual by resuspending the pellet with a pipette tip.<br>Wash the pellet three times to remove salts from the binding solution completely. |

## References


1. Vogelstein, B. and Gillespie, D., Proc. Natl. Acad. Sci. USA, 76, 615-619, 1979.
2. Ausubel, F.M., et al. Eds, Current protocols in Molecular Biology, John Wiley & Sons, Inc., NY, 1997.
3. Wilson, V.G., Bio Techniques 6, 733, 1988.
4. Smith, L.S., Lewis, T.L. and Matsui, S.M., Bio Techniques, vol. 18, 972,1995.

## SAFETY INFORMATION

Binding Buffer

Hazard-determining component of labeling: guanidinium thiocyanate

Xn Harmful

### Risk phrases

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
R32 Contact with acids liberates very toxic gas.
R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

### Safety phrases

S9 Keep container in a well-ventilated place.
S23 Do not breathe gas/fumes/vapour/spray.
S36/37 Wear suitable protective clothing and gloves.
S60 This material and its container must be disposed of as hazardous waste.
S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.