High-Q™ Spin-Column Gel Extraction & Cleanup Kit

Ordering info

TBK0190, 3 reactions (sample)

TBK0191, 50 reactions

TBK0192, 200 reactions

Description

High-Q™ Spin-Column Gel Extraction & Cleanup Kit is an easy silica-membrane-based DNA purification kit suitable for the purification of DNA embedded in agarose gels or to remove salts, proteins, nucleotides, primers or other contaminants from enzymatic reactions.

Features

- Easy and fast protocol.
- Complete removal of DNA contaminants.
- Excellent DNA recovery.
- No phenol extraction.

Applications

- DNA isolation from DNA embedded in agarose gels or enzymatic reaction mixtures such as PCR, restriction digestion, labelling reactions, dephosphorylation, etc.
- DNA obtained is suitable for downstream molecular biology applications such as cloning, PCR, sequencing, digestion, genotyping, etc.

Quality Control

Isolation of a 0.5 kb DNA fragment from 0.7% agarose gel is checked by: integrity (agarose gel electrophoresis), quantity and quality (A260/280= 1.8 ± 0.2).

Kit Components

Components	TBK0191	TBK0192
High-Q™ Spin Columns	50	200
Collection Tubes	50	200
BQ Buffer	60 mL	240 mL
BCL1 Buffer	30 mL	120 mL
BQ2 Buffer	8 mL[a]	35 mL[b]
Sodium Acetate 3M, pH 5	1 mL	1 mL
Elution Buffer	15 mL	15 mL
COBALT™ DNA Loading Buffer 6x	0.1 mL	0.5 mL

Order Info Kit Components: *High-Q™ Spin Columns (TBM0017) | Collection Tubes (TBM0020) | BQ Buffer (TBB0527) | BCL1 Buffer (TBB0535) | BQ2 Buffer (TBB0529) | Elution Buffer (TBB0510) | COBALT™ DNA Loading Buffer 6x (TBB0321).*

Before its use:

[a] Add 32 mL absolute ethanol and mix well.

[b] Add 140 mL absolute ethanol and mix well.

Storage

Store the kit at 25°C.

Material required (not supplied)

- Ethanol (CAS 64-17-5).
- Isopropanol (CAS 67-63-0).
- 1.5 mL Tubes.

PROTOCOL

I. DNA PURIFICATION FROM AGAROSE GELS

1. Cut with a clean scalpel the agarose region containing your DNA fragment. Try to eliminate all the extra agarose gel.
2. Introduce the slice gel into a pre-weight 1.5 mL tube. Weight the gel excised.

   If the weight of gel excised is higher than 400 mg, split it in two pieces and use two columns.
3. Add **BQ Buffer for each 100 mg of gel weight** as it is indicated:

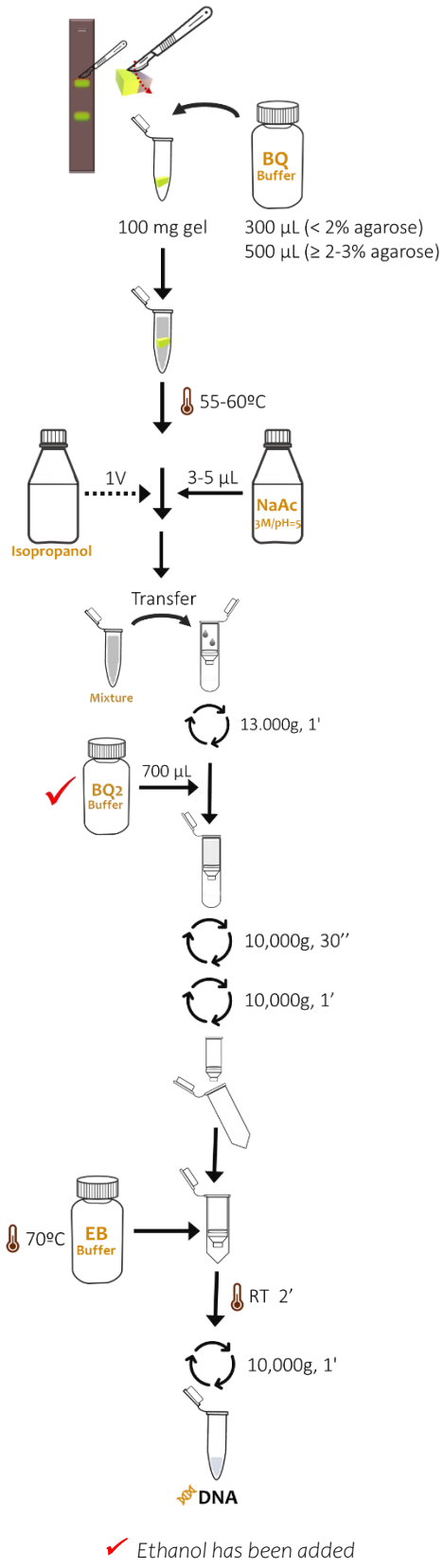
Agarose Gel Concentration	BQ Buffer Volume	Examples
< 2%	300 µL	*To 110 mg agarose gel, add 330 µL BQ Buffer*
≥ 2-- 3%	500 µL	*To 110 mg agarose gel, add 550 µL BQ Buffer*

4. Incubate at 55-60 °C and shake occasionally until agarose is completely dissolved (aprox. 10 minutes).
5. ***Optional**, if your **DNA fragment is <500 bp or >10 kb**, add **1 gel volume of isopropanol** and mix well.*
6. To guarantee optimal DNA adsorption, add **3-5 µL 3M Sodium Acetate pH=5.0** and mix well.
7. Transfer the mix to a High-Q™ Spin Column placed into a Collection Tube.

   The maximum loading volume of High-Q™ Spin Column is 700 µL. If your volume is higher repeat this step as many times as you needed.
8. Centrifuge at 10,000 g for 30 seconds. Remove the flow-through and place back High-Q™ Spin Column into a Collection Tube.
9. Add **700 µL BQ2 Buffer**.

   ✔*Check absolute ethanol has been added to BQ2 Buffer.*
10. Centrifuge at 10,000 g for 30 seconds. Remove the flow-through and place back the High-Q™ Spin Column into a Collection Tube.
11. To dry High-Q™ Spin Column, centrifuge at 10,000 g for 1 minute.
12. Place the High-Q™ Spin Column into a clean 1.5 mL Tube.
13. Add **30 - 60 µL prewarmed Elution Buffer** or **Water (Molecular Biology Grade)** to elute purified DNA.

    Prewarm Elution Buffer or Water at 70°C.
14. Incubate at room temperature, 2 minutes.
15. Centrifuge at 10,000 g for 1 minute.
16. Store DNA at -20°C.



✔ *Ethanol has been added*

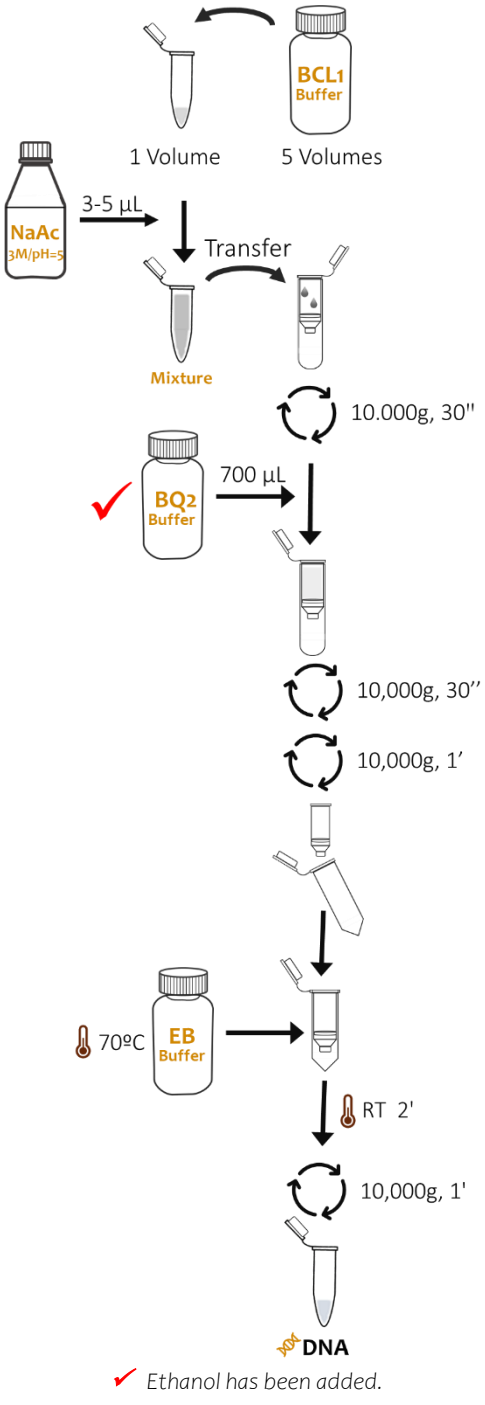
II. DNA PURIFICATION FROM ENZYMATIC REACTION

1. Add **5 volumes of BCL1 Buffer to 1 volume of DNA solution** (PCR, enzymatic reaction, etc).
2. Mix well by pipette.
3. To guarantee optimal DNA adsorption, add **3-5 µL 3M Sodium Acetate pH=5.0** and mix well.
4. Transfer the mix to a High-Q™ Spin Column placed into a Collection Tube.

   The maximum loading volume of the High-Q™ Spin Column is 700 µL. If your volume is higher repeat step 3 as many times as you needed.
5. Centrifuge at 10,000 g for 30 seconds. Remove the flow-through and place back the High-Q™ Spin column into a Collection Tube.
6. Add **700 µL BQ2 Buffer**.

   ✔*Check absolute ethanol has been added to BQ2 Buffer.*
7. Centrifuge at 10,000 g for 30 seconds. Remove the flow-through and place back the Spin column into a Collection Tube.
8. To dry High-Q™ Spin Column and eliminate residual ethanol, centrifuge again at 10,000 g for 1 minute.
9. Place the High-Q™ Spin Column into a clean 1.5 mL Tube.
10. Add **30 - 60 µL prewarmed Elution Buffer** or **Water (Molecular Biology Grade)** to elute purified DNA.

    Prewarm Elution Buffer or Water at 70°C.
11. Incubate at room temperature, 2 minutes.
12. Centrifuge at 10,000 g for 1 minute.
13. Check DNA quantity by spectrophotometry and quality on agarose electrophoresis gel using COBALT™ Loading Buffer 6x provided.
14. Store at -20°C.



✔ *Ethanol has been added.*