BIOTOOLS B&M LABS.S.A.

Manufactured by:

BIOTOOLS B&M Labs, S.A.
Valle de Tobalina - 52 - Nave 39
28021 Madrid
Spain

Tel. (34) 91 710 00 74
Fax (34) 91 505 31 18
e-mail: info@biotools.eu
www.biotools.eu

SPEEDTOOLS PCR CLEAN-UP KIT

Designed for the direct purification of DNA from PCR products or from agarose gels (TAE/TBE)

Instructions for Use

(Ref. 21.200M/1/2)

PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.

1. BASIC PRINCIPLE

SPEEDTOOLS PCR CLEAN-UP KIT is designed for a rapid and easy isolation of nucleic acids fragments from aqueous solutions (e.g.PCR) as well as from agarose or polyacrylamide gels.

DNA from PCR reactions or agarose gels binds to the silica membrane (BINDING COLUMN) in the presence of chaotropic salts from the binding BUFFER B. Contaminants like salts and soluble macromolecular components are removed by a washing step with wash BUFFER T3. Pure DNA is finally eluted under low ionic strength conditions with slightly alkaline BUFFER E or sterile bidistilled water.

Table 1. General Characteristics of the Kit	
Elution volume	15-30 µL
Binding Capacity	25 µg
Optimal recovery	<15 µg[a]
Yield	60-90%[b]
Sample material	Up to 200 µL of aqueous solutions (e.g.PCR reaction) or 200 mg of agarose gel[c]
Fragment length	From 50 bp to ~ 20 kb[d]
Time / Preparation	10 min/ 6 preps of aqueous solutions 20 min/ 6 preps of gel extractions

[a] For fragments of 100-500 bp in an elution volume of 30 µL

[b] Depending on the fragment size and elution procedure

[c] With only one loading step; more sample with additional BUFFER B and multiple loading steps

[d] The kit is recommended for DNA up 10-15 kb. Longer fragments can be purified but recovery may be low. Fragments larger than 20 kb may be mechanically damaged by the fast centrifugation through the membrane.

2. KIT CONTENTS

Speedtools PCR Clean-Up kit			
Reagents	**10 Preps Ref. 21.200M**	**50 Preps Ref. 21.201**	**250 Preps Ref. 21.202**
BUFFER B Binding Buffer with pH Indicator	10 mL	40 mL	5 x 40 mL
BUFFER T3 (concentrated) Wash Buffer	6 mL	25 mL	5 x 25 mL
BUFFER E Elution Buffer	13 mL	13 mL	5 x 13 mL
BINDING COLUMNS	10	50	5 x 50
COLLECTION TUBES	10	50	5 x 50
PROSPECT	1	1	5 x 1

3. PRODUCT DESCRIPTION

a. Kit Specifications

- The kit is designed for the purification of DNA from agarose or polyacrylamide gels and for the direct purification of PCR products.
- Primer from PCR reactions are eliminated while small DNA fragments (>50 bp) are still bound to silica membrane and they can be purified with high recovery (details in Section 5.D).
- DNA fragments from PCR reaction buffers rich in various detergents can be purified with high recovery.
- The adsorption of DNA to the column membrane is pH dependent. The pH indicator included in the Binding BUFFER B ensures optimal binding conditions with pH <7 (Section 3.b).
- Standard as well as low melting agarose gels can be used (TAE or TBE).
- The purified DNA can directly be used for hybridization, sequencing, PCR, restriction, ligation, in vitro transcription, cloning, labelling or any other kind of enzymatic reaction.

Uses of the Kit		
DNA Purification from Aqueous Solutions (e.g. PCR Products)	**YES**	**Section 5.A**
DNA Purification from Liquid Samples Containing SDS	**YES**	**Section 5.B**
Purification of Single Stranded DNA	**YES**	**Section 5.C**
Removal of Small DNA Fragments and Primer-Dimers	**YES**	**Section 5.D**
DNA Extraction from Agarose Gels (standard or low melting)	**YES**	**Section 5.E**
RNA Extraction from Agarose Gels	**YES**	**Section 5.F**
DNA Extraction from Polyacrylamide Gels	**YES**	**Section 5.G**

b. Binding BUFFER (Buffer B) with pH INDICATOR

The optimal pH to bind even small DNA fragments to the silica membrane, of the binding column, is approximately 5.0–6.0. The Binding BUFFER B is sufficiently buffered to maintain this pH for all standard PCR reaction buffers or agarose gel buffer systems. However, to be sure that the pH is right even for samples with extreme alkaline pH or high buffer concentration, a pH indicator has been added. The pH indicator does not interfere with DNA binding and is completely removed during the purification.

When you mix the sample (aqueous solutions or pieces of agarose) with the BUFFER B you can find three options:

1. ***The binding mixture remains yellow- pH of the mixture < 6.0*****:** Optimal DNA absorption and its *recovery will be the maximum*.
2. ***The binding mixture turns green- pH of the mixture between 6.0-7.0*****:** DNA absorption will be less efficient and its *recovery will be slightly affected*.
3. ***The binding mixture turns blue- pH of the mixture > 7.0*****:** DNA absorption will be inefficient and its *recovery will be drastically reduced*.

If a change in colour is observed, the pH should be corrected by adding 4 M sodium acetate pH 5.0; or small amounts of hydrochloric acid (HCl) until the mixture turns to yellow (pH □6).

The BUFFER B with pH Indicator allows:

- *Quick visualisation of optimal or no-optimal binding conditions*
- *Restore binding mixture to correct conditions*
- *Identify undissolved pieces of gel* during DNA/RNA gel extraction

c. Elution Procedures

For the elution of DNA, one of the following solutions can be used: BUFFER E, included in the kit (5 mM Tris/HCl, pH 8.5); TE buffer, pH 8.5; or sterile bidistilled water, pH 8.5.

NOTE: *EDTA in TE buffer may cause problems in subsequent reactions. The pH of the water should be checked before use to avoid lower recovery yields (make sure the pH is still >7).*

The standard elution volume is **15-30 µL** which is the best compromise for high recovery and high DNA concentration for fragments <1000 bp. With an elution volume of 15 µL of BUFFER E, a recovery of **70-95%** is usually obtained for DNA fragments between **50-10,000 bp** resulting in highly concentrated eluates. Elution from **gel extraction is 10-20 % less efficient** than elution from aqueous solutions.

If larger amounts (5-15 µg) of DNA have to be purified (e.g. from PCR reactions > 200 µL or gel slices > 200 mg), elute with at least 30 µL of BUFFER E.

To improve the DNA recovery after gel extraction and/or for large DNA fragments (> 5-10 kb), the following modifications can be applied to the standard elution protocol:

- ***Heat elution BUFFER E*** *to* 70°C and incubate this buffer on the column for 5 min at 70°C.
- *Apply elution BUFFER E to the column and centrifuge first* ***1 min at 30-50 x g*** *and then* ***1 min at 11,000 x g***
- *Do* ***2 or 3 elution steps*** *with 20-30 µL of fresh BUFFER E.*

4. STORAGE CONDITIONS AND PREPARATION OF WORKING SOLUTIONS

All kit components can be stored at room temperature (18-25°C) through the expiration date printed on the packaging label.

NOTE: *BUFFER B contains chaotropic salt. Wear gloves and goggles.*

Before starting any protocol with **SPEEDTOOLS PCR CLEAN-UP KIT** prepare BUFFER T3 as follow:

Buffer T3:

- ✓ **10 preps format:** Add **24 mL of molecular grade ethanol** (96-100%) to Buffer T3 concentrate. Mark the label of the bottle to indicate that ethanol was added.
- ✓ **50 preps format:** Add **48 mL of molecular grade ethanol** (96-100%) to Buffer T3 concentrate. Mark the label of the bottle to indicate that ethanol was added.

Wash BUFFER T3 is stable at room temperature (18-25 °C) for at least 12 months.

5. PROTOCOLS

A. Direct Purification of PCR Products

The following protocol is suitable for ***PCR clean-up as well as DNA concentration and removal of salts, enzymes, etc. from enzymatic reactions*** (SDS < 1%).

Before starting the preparation:

- Prepare Buffer T3 according to Section 4.
- Before elution and if necessary preheat Elution Buffer E to 70ºC.

STEP	DESCRIPTION		
1	**ADJUST DNA BINDING CONDITIONS** Mix **1 VOL of sample** with **2 VOL of BUFFER B** For sample volumes < 30 µL adjust the volume of the reaction mixture to 50-100 µL with water. *If the binding mixture turns green or blue, correct the pH by adding acid solutions (Section 3.b).* ***Note:*** *For removal of small DNA fragments (e.g. primer dimers) use dilutions of BUFFER B (Section 5.D).*		**1 VOL SAMPLE** **+** **2 VOL BUFFER B**
2	**BIND DNA** Use one Binding Column for each preparation and place it into a Collection Tube. Load the sample carefully. Centrifuge **30 s** at **11,000** × ***g***. Discard flow-through and place the Column back into the Collection Tube.		**Load sample into a column** *30 s,* *11,000 × g*
3	**WASH SILICA MEMBRANE** Add **700 µL BUFFER T3**. Centrifuge **30 s** at **11,000 x *g***. Discard flow-through and place the Column back into the Collection Tube. ***Note:*** *Repeat previous washing step to minimise chaotropic salt carry-over and improve A_{260}/A_{230} values.*		**+** **700 µL BUFFER T3** *30 s,* *11,000 × g* **(1 or 2 washing steps)**
4	**DRY SILICA MEMBRANE** Centrifuge **1 min** at **11,000 x *g*** to remove BUFFER T3. The column must not come in contact with the flow-through. ***Note:*** *Complete removal of ethanol can be achieved by incubating the Column for 2-5 min at 70ºC prior to elution.*		*1 min,* *11,000 × g*
5	**ELUTE PURE DNA** Place the COLUMN into a new 1.5 mL microcentrifuge tube. Add **15-30 µL** elution **BUFFER E**. Dispense buffer directly onto the silica membrane. Incubate at **room temperature** for **1 min**. Centrifuge **1 min** at **11,000 x *g***. The eluate contains the pure DNA sample. ***Note:*** *DNA recovery of larger fragments (> 1000 bp) can be increased by multiple elution steps with fresh buffer, heating to 70ºC and incubation for 5 min (Section 3.c).*		**+** **15-30 µL BUFFER E** **Incubate 1 min at RT** *1 min,* *11,000 × g*

B. DNA Purification from Liquid Samples Containing SDS (Buffer BS Ref. 21.2031[1], not included)

The following protocol is suitable for ***DNA clean-up of aqueous solutions containing SDS***, for example in applications like Chromatin Immunoprecipitation (ChIP).

Before starting the preparation:

- Prepare Buffer T3 according to Section 4.
- Before elution and if necessary preheat Elution Buffer E to 70ºC.

STEP	DESCRIPTION		
1	**ADJUST DNA BINDING CONDITIONS** Mix **1 VOL of sample** with **5 VOL of BUFFER BS**. ***Note:*** *If SDS starts to precipitate add 1 volume of isopropanol or warm sample to 20-30 °C.*		**1 VOL SAMPLE** **+** **5 VOL BUFFER BS**
2	**Proceed with Step 2 of the Protocol for Direct Purification of PCR Products (Section 5.A)**		

C. Purification of Single Stranded DNA (Buffer BD Ref. 21.204[2], not included)

The binding BUFFER B (included in the Kit) is able to bind single stranded DNA (ssDNA) > 150 bases. Shorter oligonucleotides, especially primers, are completely removed.

If you need to purify short ssDNA, the additional Binding Buffer BD can be used (**Figure 1**).

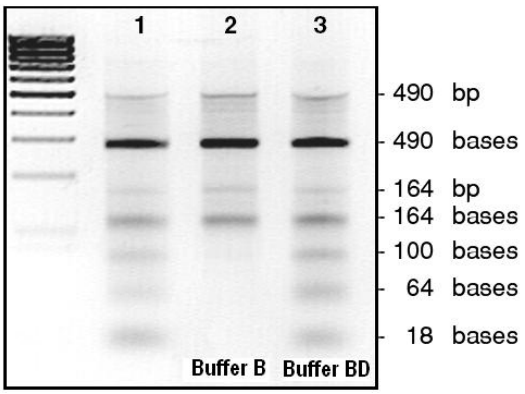
Before starting the preparation:

- Prepare Buffer T3 according to Section 4.
- Before elution and if necessary preheat Elution Buffer E to 70ºC.

STEP	DESCRIPTION		
1	**ADJUST DNA BINDING CONDITIONS** Mix **1 VOL of sample** with **2 VOL of BUFFER BD**. *If the sample contains large amounts of detergents or other critical substances, double the volume of BUFFER BD.*		**1 VOL SAMPLE** **+** **2 VOL BUFFER BD**
2	**Proceed with Step 2 of the Protocol for Direct Purification of PCR Products (Section 5.A)**		

[1] BUFFER BS has to be ordered separately Ref. 21.203
[2] BUFFER BD has to be ordered separately Ref. 21.204

Figure 1. Purification of dsDNA and ssDNA using BUFFER B and BUFFER BD.



PCR fragments, amplified using one phosphorylated and one dephosphorylated primer, were partially digested with λ-Exonuclease. Samples were purified using **BUFFER B** and **BUFFER BD** and run on a 1 % TAE agarose gel. Remaining double stranded DNA can be seen as faint bands. The corresponding ssDNA is running slightly faster due to secondary structure formation. Compared to the input DNA (lane 1), **BUFFER B** removes ssDNA < 150 bases (lane 2), whereas **BUFFER BD** leads to full recovery of even primer oligonucleotides (lane 3).

D. Removal of Small DNA Fragments and Primer-Dimers

SPEEDTOOLS PCR CLEAN-UP KIT is designed to remove even traces of unused primers, primer-dimers or non-specific PCR products that might interfere with your downstream sequencing or cloning applications.

Removal of double stranded DNA >50 bp can be achieved by diluting an aliquot of BUFFER B with sterile bidistilled water in an appropriate ration and then proceed with **Step 2 of the Protocol for Direct Purification of PCR Product** (Section 5.A).

Diluting BUFFER B in a certain range lowers the binding efficiency for small fragments without compromising the recovery of larger PCR products (**Figure 2**).

The dilution ratio to choose depends on the ***fragment size*** to be purified as well as on the ***PCR buffer system*** used. The appropriate ratio of BUFFER B dilution should be determined in advance.

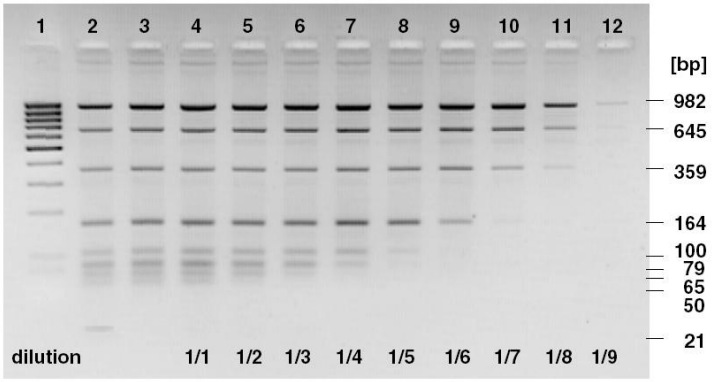
Influence of DNA fragment size: The smaller the fragment to be removed, the less you have to dilute BUFFER B.

Influence of PCR buffer system: The influence of the PCR buffer system on the removal of small fragments is more complex. Some reaction buffers contain detergents like Tween or additives like betaine which lower the melting temperature of the DNA template. These substances are usually found in PCR buffers for high fidelity or long range PCR, they tend to decrease the binding efficiency of DNA to the silica membrane; therefore their presence have to be considered when choosing a dilution ratio of BUFFER B.

Therefore for each size of small fragments >50 bp that has to be removed; and for each buffer system, determine in advance the appropriate ratio of BUFFER B dilution. *As a rule of thumb if a PCR buffer system without special additives is used, adding **3 to 5 VOL of WATER** to **1 VOL of BUFFER B** will lead to removal of small fragments up to 100 bp. Otherwise, if PCR buffer includes additives adding **1 to 3 VOL of WATER** to **1 VOL of BUFFER B** will be sufficient.*

Figure 2 shows a purification result with dilution series of BUFFER B. Non diluted BUFFER B (lane 3), as well as BUFFER B plus one volume of water (lane 4), lead to 100% recovery of a PCR fragments (lane 2). Use of more diluted BUFFER B cuts off more and more of the low molecular mass bands. Usually a dilution with 5 volumes of sterile bidistilled water should be sufficient to eliminate even larger unwanted primer-dimer fragments while purifying the 164 bp fragment with >90% (lane 8).

Figure 2. Purification of PCR reactions using dilutions of Binding Buffer B.



Lane 1: 100 bp Ladder Marker (BIOTOOLS Ref. 31.006)
Lane 2: Sample (21 base primer, 50, 65, 79, 100, 164, 359, 645 and 982 bp fragments)
Lane 3: Purification with 100% BUFFER B
Lane 4-12: Purification using diluted BUFFER B with 1-9 volumes of sterile bidistilled water.

Extraction and Purification of Nucleic Acids from Gels

Buffer system: Speedtools PCR CLEAN-UP KIT is compatible with TAE and TBE electrophoresis buffers. However, it is preferred to use fresh TAE buffer over TBE for preparative agarose gels. TAE does not interact with agarose, resulting in a higher DNA yields.

Running conditions: The temperature during electrophoresis should be low in order to increase the resolution of the DNA separation and avoid melting of the gel, thus causing denaturation of the DNA. Use fresh buffer and run the gel at low voltage (60V), for as short as possible. As soon as the DNA band of interest is sufficiently separated from the rest, stop the gel and cut out the band.

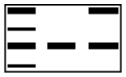
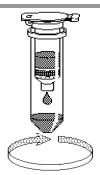
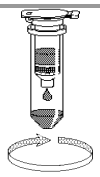
Exposition to UV light: Exposure the gel to UV light as short as possible. Prolonged exposure can damage the DNA. Wear gloves and a face mask to protect your skin and eyes from UV light.

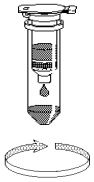
Cutting out the band: Make sure to cut through the gel vertically and remove all excess. Up **to 200 mg of agarose gel** can be dissolved with **400 µL of BUFFER B** and load on the column in one step. More than 200 mg can be load by increasing BUFFER B proportionally and adding multiple loading steps. Use **0.7-1.0 % agarose** gels rather than higher percentages.

E. DNA Extraction and Purification from Agarose Gels

Before starting the preparation:

- Set an incubator or water bath to 50 ºC.
- Prepare Buffer T3 according to Section 4.
- Before elution and if necessary preheat Elution Buffer E to 70ºC.

STEP	DESCRIPTION		
1	**EXCISE DNA FRAGMENT** Take a clean scalpel to excise the DNA fragment from an agarose gel. Minimise the gel volume. ***Determine the weight of the gel slice and transfer it to a clean tube.*** ***Note:*** *Minimise UV exposure time to avoid damaging the DNA.*		**Excise Gel Band**
2	**GEL LYSIS** For each **100 mg of agarose** gel <2% add **200 µL BUFFER B.** *If the binding mixture turns green or blue correct the pH by adding acid solutions (Section 3.b).* Incubate sample at **50ºC** until the gel slices are dissolved (**5-10 min**). Vortex briefly every 2-3 min. ***Note:*** *For gels containing > 2% agarose, double the volume of BUFFER B.*		**100 mg gel** **+** **200 µL BUFFER B** **50ºC, 5-10 min**
3	**BIND DNA** For each preparation, take one Binding Column and place it into a 2 mL Collection Tube. Load the sample carefully. Centrifuge **30 s** at **11,000 × *g***. Discard flow-through and place the Column back into the Collection Tube. *Load remaining sample if necessary and repeat the centrifugation step.*		**Load sample into a column** *30 s, 11,000 × g*
4	**WASH SILICA MEMBRANE** Add **700 µL BUFFER T3**. Centrifuge **30 s** at **11,000 x *g***. Discard flow-through and place the Column back into the Collection Tube. ***Note:*** *Repeat previous washing step to minimise chaotropic salt carry-over and improve A_{260}/A_{230} values.*		**+** **700 µL BUFFER T3** *30 s, 11,000 × g* **(1 or 2 washing steps)**
5	**DRY SILICA MEMBRANE** Centrifuge **1 min** at **11,000 x *g*** to remove BUFFER T3. The column must not come in contact with the flow through. ***Note:*** *Complete removal of ethanol can be achieved by incubation of the Column for 2-5 min at 70ºC prior to elution.*		*1 min, 11,000 × g*

6	**ELUTE PURE DNA** Place the COLUMN into a new 1.5 mL tube. Add **15-30 µL BUFFER E**. Dispense buffer directly onto the silica membrane. Incubate **at room temperature** for **1 min**. Centrifuge **1 min** at **11,000 x *g***. The eluate contains the pure DNA sample. ***Note:*** *DNA recovery of larger fragments (> 1000 bp) can be increased by multiple elution steps with fresh buffer, heating to 70ºC and incubation for 5 min (Section 3.c).*		**+** **15-30 µl BUFFER E** **Incubate RT 1 min** *1 min,* *11,000 × g*

F. RNA Extraction and Purification from Agarose Gels (Buffer BD Ref. 21.204[3], not included)

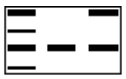
Not only DNA but also RNA can be extracted from agarose gels. To efficiently bind especially the small, single stranded RNA, **BUFFER BC** has to be used instead of standard binding BUFFER B.

To fractionate RNA, run a standard RNA gel with denaturing RNA loading buffer, but **do not use formaldehyde or glyoxal.** These compounds not only inactivate RNases and denature RNA, but also modify RNA. As a result, the RNA yield is significantly reduced and more important the RNA may not work properly in enzymatic downstream applications, such as RT-PCR or in vitro transcriptions.

Without formaldehyde, the RNA is very sensitive to RNases effect. Use gloves and make sure all equipment is RNase-free, especially the agarose, and the running buffers. Run the gel as short and as cold (low voltage, cold room) as possible. Note that the RNA may form secondary structures and may run differently from denaturing agarose gels.

Before starting the preparation:

- Set an incubator or water bath to 50 ºC.
- Prepare Buffer T3 according to Section 4.
- Before elution and if necessary preheat Elution Buffer E to 70ºC.

STEP	DESCRIPTION		
1	**EXCISE DNA FRAGMENT** Take a clean scalpel to excise the RNA fragment from an agarose gel. Remove all excess agarose. ***Determine the weight of the gel slice and transfer it to a clean tube.*** ***Note:*** *Minimise UV exposure time to avoid damaging the RNA.*		**Excise Gel Band**
2	**GEL LYSIS** For each **100 mg of agarose** gel <2% add **200 µL BUFFER BD.** Incubate sample at **50ºC** until the gel slice is completely dissolved (**5-10 min**). Vortex briefly every 2-3 min. ***Note:*** *For gels containing > 2% agarose, double the volume of BUFFER BD.*		**100 mg gel** **+** **200 µL BUFFER BD** **50ºC, 5-10 min**
3	**Proceed with Step 3 of the Protocol for DNA Extraction and Purification from Agarose Gels (Section 5.E)**		

[3] BUFFER BD has to be ordered separately Ref. 21.204

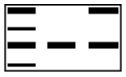
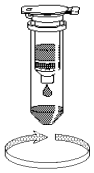
G. DNA Extraction and Purification from Polyacrylamide Gels

In polyacrylamide gels, the acrylamide monomers are covalently linked in a chemical reaction, Therefore, the gel cannot be dissolved like agarose gels to extract the trapped DNA.

Polyacrylamide gels are usually extracted by the "crush and soak" method where a small piece of gel is crushed and incubated in a diffusion buffer. The DNA is then allowed to passively diffuse out of the gel and is then purified from the diffusion buffer. The **diffusion buffer** (500 mM ammonium acetate pH 8.0; 0.1 % SDS; 1 mM EDTA; 10 mM magnesium acetate) is not provided with the kit.

Before starting the preparation:

- Set an incubator or water bath to 50 ºC.
- Prepare Buffer T3 according to Section 4.
- Before elution and if necessary preheat Elution Buffer E to 70ºC.

STEP	DESCRIPTION		
1	**EXCISE DNA FRAGMENT** Take a clean scalpel to excise the DNA fragment from the acrylamide gel. Minimise the gel volume. ***Determine the weight of the gel slice and transfer it to a clean tube.*** ***Note:*** *Minimise UV exposure time to avoid damaging the DNA.*		**Excise Gel Band**
2	**CRUSH GEL** Crush the gel slice using a disposable pipette tip with a melted end to resemble a pestle for the microcentrifuge tube "mortar". The smaller the pieces, the better the DNA recovery.		**Crush gel slice**
3	**EXTRACT DNA** Add **200 µL** of **diffusion buffer** to each **100 mg** of crushed gel. Make sure that all gel pieces are submerged in the buffer. Incubate for **30-60 min** at **50ºC** or **over night** at **37ºC**		**100 mg gel** **+** **200 µL Diffusion Buffer** **50ºC, 30-60 min** (or O/N at 37 ºC)
4	**REMOVE POLYACRYLAMIDE** Centrifuge **1 min** at **14,000 x *g*** to pellet the polyacrylamide and transfer the supernatant to a new microcentrifuge tube. Alternatively, transfer the mixture to a Column and centrifuge **1 min** at **14,000 x *g*** to retain the gel on the column. ***Keep the flow-through which contains the DNA*** ***Optional:*** *To increase the final yield, repeat step 3 and 4 and combine both supernatants or flow-throughs.*	 	*1 min, 14,000 × g* *Recovery supernatant* *Or* *Transfer to column* *1 min, 14,000 × g* *Recovery flow-through*
5	**ADJUST DNA BINDING CONDITIONS** Mix **1 VOL of sample** with **2 VOL of BUFFER B** *If the binding mixture turns green or blue, correct the pH by adding acid solutions (Section 3.b).* ***Note:*** *To obtain higher yields for small fragments (□ 50 bp) add 2 VOL of molecular grade ethanol.*		**1 VOL SAMPLE** **+** **2 VOL BUFFER B**
6	**Proceed with Step 2 of the Protocol for Direct Purification of PCR Products (Section 5.A)**		

6. TROUBLESHOOTING

Problem	Possible cause and suggestions
Low DNA yield	***Reagents not prepared properly*** • Add indicated volume of 96-100% molecular grade ethanol to BUFFER T3 concentrate and mix (Section 4). ***Binding Mixture pH > 6.0*** • If the binding mixture turns green or blue, correct the pH by adding acid solutions (Section 3.b). ***Incomplete dissolving of gel slice*** • Increase incubation time or add two volumes of BUFFER B, vortex the tube every 2 minutes during the incubation at 50ºC. The Buffer B with pH indicator allows identify un-dissolved pieces of gel, preventing loss of DNA during the process. ***Insufficient drying of the column membrane*** • Centrifuge 5 min at 11,000 x g and/or incubate column for 2-5 min at 70ºC before elution to remove ethanolic BUFFER T3 completely. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). ***Incomplete elution*** • Especially when larger amounts of DNA (> 5 µg) or long DNA fragments (> 1000 bp) are bound to column, do multiple elution steps with fresh BUFFER E, pre-warm this buffer to 70°C, and incubate the silica membrane at room temperature for 5 min before centrifugation (Section 3.c).
Incomplete lysis of agarose slices	***High amount of agarose*** • Use doubled volumes of BUFFER B for highly concentrated agarose gels. ***Insufficient time and temperature*** • Check incubation temperature. Incubation can be prolonged up to 20 min; vortex every 2 min. The Buffer B with pH indicator allows identify un-dissolved pieces of agarose, preventing loss of DNA during the process. • Large gel slices can be quenched or crushed before addition of BUFFER B to shorten the melting time.
Suboptimal performance of DNA in NanoDrop® Spectophotometer Analysis	***Carry-over of traces of silica particles*** • NanoDrop® Spectrophotometer technology is very sensitive to any particles included in the sample. To pellet the silica particles centrifuge > 2min at 11,000 x g and take the supernatant for further use. ***Carry-over of chaotropic salts (low ratio A_{260}/A_{230})*** • Perform a second washing step with BUFFER T3 in case of sensitive downstream applications to remove traces of BUFFER B.
Suboptimal performance of DNA in sequencing, restriction or ligation reactions	***Carry-over of ethanol/ethanolic BUFFER T3*** • Centrifuge 5 min at 11,000 x g and/or incubate the column for 5-10 min at 70ºC before elution to remove ethanolic BUFFER T3 completely. Ethanolic contaminations are also indicated by gel loading problems (samples float out of gel slots). ***Carry-over of chaotropic salts*** • Perform a second washing step with BUFFER T3 in case of sensitive downstream applications to remove traces of BUFFER B. ***Elution of DNA with buffers other than BUFFER E (e.g. TE Buffer)*** • EDTA might inhibit sequencing reactions. Repurify DNA and elute in BUFFER E or sterile bidistilled water. Take care with the pH of water (Section 3.c). ***Not enough DNA used for sequencing reaction*** • Quantify DNA by agarose gel electrophoresis before setting up sequencing reactions. ***DNA was damaged by UV light*** • Reduce UV exposure time to a minimum when excising a fragment from an agarose gel.

7. ORDERING INFORMATION

SPEEDTOOLS KIT	10 PREPS	50 PREPS	250 PREPS
SPEEDTOOLS **DNA EXTRACTION** KIT	Ref. 21.130M	Ref. 21.131	Ref. 21.132
SPEEDTOOLS **TISSUE DNA EXTRACTION** KIT	Ref. 21.135M	Ref. 21.136	Ref. 21.137
SPEEDTOOLS **RNA VIRUS EXTRACTION** KIT	Ref. 21.140M	Ref. 21.141	Ref. 21.142
SPEEDTOOLS **FOOD DNA EXTRACTION** KIT	Ref. 21.175M	Ref. 21.176	Ref. 21.177
SPEEDTOOLS **PLANT DNA EXTRACTION** KIT	Ref. 21.170M	Ref. 21.171	Ref. 21.172
SPEEDTOOLS **TOTAL RNA EXTRACTION** KIT	Ref. 21.210M	Ref. 21.211	Ref. 21.212
SPEEDTOOLS **PCR CLEAN-UP** KIT	Ref. 21.200M	Ref. 21.201	Ref. 21.202
SPEEDTOOLS **PLASMID DNA PURIFICATION** KIT	Ref. 21.220M	Ref. 21.221	Ref. 21.222

8. PRODUCT USE RESTRICTION AND WARRANTY

1. Product for research purposes and *in vitro uses* only.
2. No claim or representation is intended for its use to identify any specific microorganism or for clinical use (diagnostic, prognosis, therapeutic, or blood bank).
3. It is rather the responsibility of the user to verify the use of the kit for a specific application range as the performance characteristic of this kit has not been verified for a specific microorganism.
4. The kit includes documentation stating specifications and other technical information. Follow the instructions in order to obtain highly pure DNA fragments.
5. BIOTOOLS warrants meeting the stated specifications of the kit. Any product not fulfilling the specifications included in the product sheet will be replaced.
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