GenUP™ PCR/Gel Cleanup Kit

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

PRODUCT	GenUP™ PCR/Gel Cleanup Kit		
CAT. NO.	BR0700501	BR0700502	BR0700503
SIZE	10 preps	50 preps	250 preps
COMPONENTS			
Buffer DISSOLVING	7 ml	40 ml	180 ml
Buffer OPTIMIZATION	5 ml	5 ml	15 ml
Buffer BINDING BP	7 ml	30 ml	150 ml
Buffer WASH B (concentrate)	6 ml (add 24 ml ethanol)	16 ml (add 64 ml ethanol)	2 × 36 ml (add 144 ml ethanol)
Buffer ELUTION	2 ml	2 × 2 ml	15 ml
Mini Filters (green)	10	50	5 × 50
Collection Tubes (2 ml)	10	50	5 × 50
Elution Tubes (1.5 ml)	10	50	5 × 50

STORAGE

Room temperature (until expiry date – see product label).
If precipitation appears, gently warm the solution to dissolve the precipitate.

FEATURES

- Dual performance kit for both PCR product clean-up and DNA purification from agarose gels
- Fast and simple procedure
- High DNA recovery yields

APPLICATIONS

- Fast purification of DNA from agarose gels
- Fast purification of products from PCR amplification reactions

DESCRIPTION

biotechrabbit™ GenUP PCR/Gel Cleanup Kit has been specially developed for a quick and easy cleanup or concentration of PCR fragments from reaction mixtures as well as extraction of DNA from both TAE and TBE agarose gels. The DNA is bound to a Mini Filter using a novel buffer, washed and then eluted in a separate tube. The purified DNA is ready to be used in all demanding molecular biology applications, including restriction digestion, ligation, sequencing, transfection into mammalian cells and in vitro transcription.

SPECIFICATIONS

STARTING MATERIAL	PCR reaction mixtures (up to 50 µl)	TAE or TBE agarose gel (up to 300 mg)
EXTRACTION TIME	Approximately 3 min	Approximately 20 min
BINDING CAPACITY	>20 µg DNA	>20 µg DNA
DNA SIZE	60 bp – 30 kb	100 bp – 30 kb
RECOVERY RATE	60–95%	60–90%

MATERIALS SUPPLIED BY THE USER

- 96–99.8% ethanol
- Centrifugation tubes
- Pipet tips

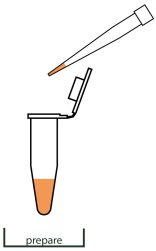
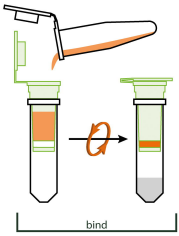
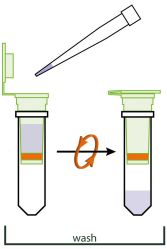
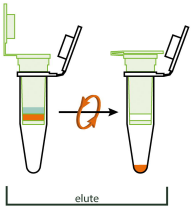
STEPS BEFORE STARTING

- Add the following volume of 96–99.8% ethanol to each bottle Buffer WASH B, close firmly, mix thoroughly and store at room temperature.

CAT. NO.	CONCENTRATE	ETHANOL	FINAL VOLUME
BR0700501	6 ml	24 ml	30 ml
BR0700502	16 ml	64 ml	80 ml
BR0700503	36 ml	144 ml	180 ml

- Mark all vials and filters to avoid confusion when purifying multiple preps.
- Perform all centrifugation steps at room temperature.
- Heat thermal mixer or water bath to 50°C.
- Optional: to increase the yield, warm the Buffer ELUTION to 50°C before use.

SHORT PROTOCOL

STEPS	SCHEME
• **PCR products only**: Transfer the PCR sample to a centrifugation tube, add Buffer BINDING BP and mix well.	
• **Agarose gel only**: Cut the gel slice containing the DNA fragment, transfer to a reaction tube. Add Buffer DISSOLVING and incubate.	
• Transfer the sample to the Mini Filter (green) placed in a Collection Tube and centrifuge.	
• Wash Mini Filter with Buffer WASH B and centrifuge.	
• Add Buffer ELUTION and centrifuge. • Purified DNA in the Elution Tube is ready for use.	

PROTOCOL FOR PURIFYING PCR PRODUCTS

PROCEDURE	NOTES
• Transfer up to 50 µl of the PCR sample to a centrifugation tube. • For PCR samples >50 µl, split into aliquots and transfer to multiple tubes. • To each tube, add 500 µl Buffer BINDING BP and mix well.	• *Alternatively,* pipet 500 µl Buffer BINDING BP directly into a Mini Filter placed in a Collection Tube and add up to 50 µl of the PCR sample. Mix by carefully pipetting three times up and down. Don't destroy the filter membrane with the pipet tip.
• Place a Mini Filter (green) in a Collection Tube. • Transfer the sample to the Mini Filter. • Centrifuge for 2 min at 10,000 × *g* (12,000 rpm). • For split PCR samples discard the filtrate and reload the Mini Filter with the remaining mixture and repeat centrifugation. • Discard the Collection Tube with the filtrate.	• Avoid any contact of the Mini Filter with the filtrate.
• Place the Mini Filter into a new 1.5 ml Elution Tube. • Add 10–20 µl Buffer ELUTION to the center of the Mini Filter. • Incubate for 2 min at room temperature. • Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min. • Discard the Mini Filter.	• Using less Buffer ELUTION than the starting PCR volume concentrates the DNA sample. • To improve yields, warm Buffer Elution to 50°C. • Longer incubation (5 min) improves yields.
• The DNA in the Elution Tube can be used immediately.	• Store the DNA at 4°C (short-term) or –20°C (long-term).

PROTOCOL FOR EXTRACTING DNA FROM AGAROSE GELS

PROCEDURE	NOTES
• Cut the DNA fragment from the agarose gel with a scalpel, shred the gel slice and transfer the pieces to a reaction tube. • Add 650 µl Buffer DISSOLVING. • Incubate at 50°C for 10 min with shaking until the agarose gel slice is completely dissolved.	• The gel slice should be a maximum of 300 mg. • Use a shaking platform (thermomixer, water bath or other rocking platform) to ensure continuous shaking during the incubation. Alternatively, vortex the sample a few times during the incubation.
• Add 50 µl Buffer OPTIMIZATION, and mix the suspension by vortexing or pipetting up and down.	
• Apply the sample to a Mini Filter (green) placed in a Collection Tube. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection tube.	• If the solution has not completely passed through the Mini Filter, centrifuge again at higher speed or prolong the centrifugation time.
• Return the Mini Filter to the Collection Tube. • Add 700 µl Buffer WASH B. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection tube.	• Before use, prepare Buffer WASH B as described above.
• Return the Mini Filter to the Collection Tube. • Add 700 µl Buffer WASH B. • Centrifuge at 10,000 × *g* (12,000 rpm) for 1 min. • Discard the filtrate and re-use the Collection tube.	
• Centrifuge at maximum speed for 2 min to remove residual ethanol. • Discard the Collection Tube.	
STANDARD ELUTION VOLUME • Place the Mini Filter into a 1.5 ml Elution Tube. • Add 30–50 µl Buffer ELUTION to the center of the Mini Filter. • Incubate for 1 min at room temperature. • Centrifuge at 6,000 × *g* (8,000 rpm) for 1 min. • Discard the Mini Filter.	• To improve yields, warm Buffer Elution to 50°C. • Longer incubation (5 min) improves yields. • To improve yields, perform elution twice using ½ volume of Buffer ELUTION.
MINI ELUTION VOLUME • Place the Mini Filter into a 1.5 ml Elution Tube. • Add 10–20 µl Buffer ELUTION to the center of the Mini Filter. • Incubate for 2 min at room temperature. • Centrifuge at 8,000 × *g* (10,000 rpm) for 1 min. • Discard the Mini Filter.	• Using less Buffer ELUTION than the starting PCR volume concentrates the DNA sample. • To improve yields, warm Buffer Elution to 50°C. • Longer incubation (5 min) improves yields.
• The Elution Tube contains the DNA.	• Store the DNA at 4°C (short-term) or –20°C (long-term).

TROUBLESHOOTING FOR PCR PRODUCTS

PROBLEM	SOLUTION
LOW RECOVERY	
Poor elution	Add the Buffer ELUTION directly to the center of the Mini Filter.
Starting volume too large	Do not transfer more than 50 µl PCR sample mixed with 500 µl Buffer BINDING BP to the Mini Filter before centrifuging. For larger PCR sample volumes, transfer multiple aliquots of 50 µl to new tubes before mixing with Buffer BINDING BP. Centrifuge after adding each aliquot to the filter.

TROUBLESHOOTING FOR AGAROSE GELS

PROBLEM	SOLUTION
LOW RECOVERY	
Buffer WASH B prepared incorrectly	Prepare Buffer WASH B exactly as described above. Store it with firmly fixed cap at room temperature.
Poor elution of DNA	Add the Buffer ELUTION directly onto the center of the Mini Filter.
Undisolved agarose gel slice	The gel slice must be completely dissolved before applying the mixture to the Mini Filter. Prolong incubation time at 50°C.
Buffer OPTIMIZATION not added	Ensure the appropriate amount of Buffer OPTIMIZATION is added to the solubilized suspension.
PROBLEMS WITH DOWN-STREAM APPLICATION, E.G. LIGATION	
Contamination with salt	Wash the Mini Filter as described above.
Contamination with agarose	Wash the Mini Filter once with Buffer DISSOLVING.
Ethanol carried over to DNA solution	Ensure the centrifugation steps are performed as described above. If the washed Mini Filter smells of ethanol, extend the centrifugation time before elution.

SAFETY PRECAUTIONS

- This kit is made for single use only!
- Don't eat or drink components of the kit!
- The kit shall only be handled by educated personal in a laboratory environment!
- Wear gloves while handling these reagents, and avoid skin contact! In case of contact, flush with water immediately!
- Handle and discard waste according to local safety regulations!
- Do not add bleach or acidic components to the waste after sample preparation!
- Buffer DISSOLVING contains guanidine isothiocyanate, which is harmful to health. Contact with acids liberates very toxic gas!

CERTIFICATE OF ANALYSIS

The components of the kit were tested by (1) purification of DNA from amplification reactions and subsequent analysis of the recovered DNA and (2) purification and recovery of DNA fragments from agarose gel and subsequent analysis of the recovered DNA.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter: http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

CONTACT BIOTECHRABBIT

biotechrabbit GmbH
Volmerstr. 9a
12489 Berlin, Germany

info@biotechrabbit.com
support@biotechrabbit.com
www.biotechrabbit.com

Phone: +49 30 555 7821-10
Fax: +49 30 555 7821-99

Certified Quality-System mdc ISO 13485 ISO 9001

Legal Disclaimer and Product Use Limitation

Purchase of product does not include a license to perform any patented applications; therefore it is the sole responsibility of users to determine whether they may be required to engage a license agreement depending upon the particular application in which the product is used. This product was developed, manufactured, and sold for in vitro use only. It is not suitable for administration to humans or animals.

Trademarks: biotechrabbit™, GenUP™ (biotechrabbit GmbH).

valid from 24.08.2016