GENECLEAN® Turbo Kit

For purification of DNA fragments of sizes 0.1 kb to 300 kb from agarose gels, PCR reactions and other enzymatic solutions

Cat. No. **111102000, 111102200, 111102400, 111102600**

Size: **10 preps, 50 preps, 100 preps, 300 preps**

Storage: **Ambient temperature (15-30 °C)**

Revision Date: **2021-01**

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1. INTRODUCTION

The GENECLEAN Turbo Kit is an advanced adaptation of the original GENECLEAN kit containing the patented GLASSMILK™ nucleic acid binding matrix. GENECLEAN Turbo Cartridge system is designed to further simplify the purification process. This system contains a special GLASSMILK embedded membrane optimized for DNA purification from any type of agarose gel or solution in the size range of 0.1 kb to 300 kb. DNA is eluted in 30 µL of H_2O or TE buffer. Turbo Cartridges also have a luer lock fitting for use with any vacuum manifold.

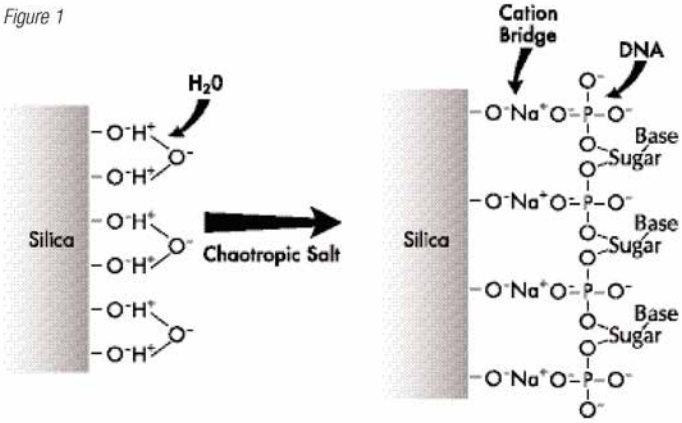
1.1 Applications for GENECLEAN Turbo Technology

- Desalting
- Isolate nucleic acids from agarose gels
- Eliminate proteins from enzymatic reactions
- Remove primers and unincorporated nucleotides from enzymatic reactions
- Separate linearized from uncut vectors
- Isolate PCR product away from genomic DNA and primers
- Clean DNA before sequencing, transfection, transformation and microinjection

1.2 How Does GENECLEAN® Technology Work?

DNA generally binds to silica at high concentrations of chaotropic salt and elutes when the salt concentration is reduced. The mechanism of DNA binding to silica in high salt has not been completely described, but may involve chaotropic salt disruption of the water structure around negatively charged silica, allowing a cation bridge to form between it and the negatively charged phosphate backbone of DNA (see figure 1). When the salt concentration is lowered, rehydration of the silica matrix breaks the attraction between the matrix and DNA. The fact that DNA binds in high salt and elutes in low salt makes this method especially useful as a purification procedure. Since the DNA is eluted with either water or a low salt buffer, it can be used immediately in subsequent reactions without precipitation or other further manipulation. This is unlike ion exchange methods that require binding in low salt and elution in high salt and require precipitation or other means of removing salt before the DNA can be used.

Figure 1



2. KIT COMPONENTS AND USER SUPPLIED MATERIALS

2.1 GENECLEAN Turbo Kit Components

Component Name	Cat. No.	Description
GENECLEAN Turbo Salt Solution	111102001 (14 mL) 111102201 (55 mL) 111102401 (110 mL) 111102601 (330 mL)	Specially prepared aqueous solution of a guanidine chaotropic binding salt that allows the DNA to bind to the GLASSMILK embedded in the GENECLEAN TurboCartridge.
GENECLEAN Turbo Wash Concentrate	111102002 (1.5 mL) 111102202 (6 mL) 111102402 (11 mL) 111102602 (32 mL)	Concentrated, proprietary salt solution to which ethanol is added to make GENECLEAN Turbo Wash (see Section 3.1). Store prepared GENECLEAN Turbo Wash at the bench (15-30 °C). Keep tightly capped to prevent evaporation of ethanol.
GENECLEAN Turbo Cartridges	111102003 (10 tubes) 111102203 (50 tubes) 111102403 (100 tubes) 111102603 (300 tubes)	Contain a specially designed composite membrane that incorporates GLASSMILK as an integral component. The irregular shape of GLASSMILK, in addition to the thickness of the filter, provides a very large surface area and greater binding capacity than most silica-based filter media. Each column can bind up to 10 µg of DNA. GENECLEAN Turbo Cartridges have a luer lock fitting so they can also be used in a vacuum manifold.
GENECLEAN Turbo Catch Tubes	111102004 (10 tubes) 111102204 (50 tubes) 111102404 (100 tubes) 111102604 (300 tubes)	1.5 mL microcentrifuge tubes with removable caps. They are used to recover and store DNA eluted from the GENECLEAN Turbo Cartridges.
GENECLEAN Turbo Elution Solution	111102005 (1 mL) 111102205 (3.3 mL) 111102405 (6.6 mL) 111102605 (20 mL)	RNase/DNase/pyrogen-free water for elution of DNA from the GENECLEAN Turbo Cartridges.
GENECLEAN Turbo GNomic Salt Solution	111102206 (14 mL) 111102406 (28 mL) 111102606 (83 mL)	Specially prepared aqueous solution of guanidine chaotropic binding salt optimized for the purification of genomic-sized DNA.

2.2 User Supplied Materials

- Benchtop microcentrifuge
- 200 proof (100%) ethanol
- Water bath or heat block
- Vacuum manifold (optional)

3. IMPORTANT CONSIDERATIONS BEFORE USE

3.1 Preparation of GENECLEAN Turbo Wash

Before first use, add the correct volume of 200 proof (100%) ethanol to the GENECLEAN Turbo Wash Concentrate according to the chart below and mix well. Do not use denatured alcohol as it can cause precipitation of salts. Label the container and store tightly capped at 15-30 °C.

Cat. No.	No. of Preps	Turbo Wash Concentrate	200 proof (100%) Ethanol
111102000	10	1.5 mL	13.5 mL
111102200	50	6 mL	54 mL
111102400	100	11 mL	99 mL
111102600	300	32 mL	288 mL

3.2 Binding Capacity

Each column can bind up to 10 µg of either ssDNA or dsDNA.

3.3 Yield Measurements

The best method for checking yields of DNA isolated by GENECLEAN Turbo is to run an aliquot on an agarose gel using known quantities in adjacent lanes as controls. OD_{260} and fluorescent readings can also be used to estimate yields, but these methods can be affected by trace amounts of salts and silica matrix. It is best to confirm these readings by gel analysis.

3.4 Agarose Types

Low-melt agarose is not required for any GENECLEAN-based kit. The procedure will work with any molecular biology-grade agarose.

3.5 Purifying Genomic DNA

Use the GENECLEAN Turbo GNomic Salt Solution for maximum binding and recovery of genomic-sized DNA.

4. SIMPLIFIED PROTOCOLS FOR EXPERIENCED USERS

4.1 Rapid Isolation of DNA from PCR Reactions & Other Enzymatic Solutions

1. Measure DNA solution volume and place in 1.5 mL microcentrifuge tube.
2. Add 5 volumes GENECLEAN Turbo Salt Solution and mix.
3. Transfer <600 µL DNA/Salt solution to GENECLEAN Turbo Cartridge placed inside a cap-less Catch Tube.
4. Centrifuge for 5 seconds until all liquid has passed through the filter. Empty Catch Tube as needed.
5. Add 500 µL prepared GENECLEAN Turbo Wash Solution to the filter.
6. Centrifuge for 5 seconds. Empty Catch Tube as needed.
7. Centrifuge the GENECLEAN Turbo Cartridge for an additional 4 minutes to remove residual Wash Solution.
8. Remove cap from a new, clean Catch Tube and insert GENECLEAN Turbo Cartridge containing bound DNA.
9. Add 30 µL GENECLEAN Turbo Elution Solution directly onto GLASSMILK embedded membrane and incubate at room temperature for 5 minutes.
10. Centrifuge for 1 minute to transfer eluted DNA to GENECLEAN Turbo Catch Tube. Discard GENECLEAN Turbo Cartridge and cap the Catch Tube.

4.2 Rapid Isolation of DNA from Agarose Gels

1. Place gel slice in 1.5 mL microcentrifuge tube.
2. Add 100 µL GENECLEAN Turbo Salt Solution per 0.1 g gel slice and mix.
3. Incubate at 55 °C for 5 minutes to melt gel. Invert tube to mix.
4. Transfer <600 µL DNA/Salt solution to GENECLEAN Turbo Cartridge placed inside a capless Catch Tube.
5. Centrifuge for 5 seconds until all liquid has passed through the filter.
6. Add 500 µL prepared GENECLEAN Turbo Wash Solution to the filter.
7. Centrifuge for 5 seconds. Empty Catch Tube as needed.
8. Centrifuge the GENECLEAN Turbo Cartridge for an additional 4 minutes to remove residual Wash Solution.
9. Remove cap from a new, clean Catch Tube and insert GENECLEAN Turbo Cartridge containing bound DNA.
10. Add 30 µL GENECLEAN Turbo Elution Solution directly onto GLASSMILK embedded membrane and incubate at room temperature for 5 minutes.
11. Centrifuge for 1 minute to transfer eluted DNA to GENECLEAN Turbo Catch Tube. Discard GENECLEAN Turbo Cartridge and cap the Catch Tube.

5. DETAILED PROTOCOLS

5.1 Rapid Isolation of DNA from PCR Reactions & Other Enzymatic Solutions

1. Measure DNA solution volume and place in a 1.5 mL microcentrifuge tube.

   NOTE ▸ *If purifying more than 10 µg of DNA, divide sample into multiple preps.*

2. Add 5 volumes of GENECLEAN Turbo Salt Solution to the DNA solution and mix by tapping the side of the tube with a finger.

   NOTE ▸ *If purifying genomic DNA, add 5 volumes of GENECLEAN Turbo GNomic Salt Solution (instead of GENECLEAN Turbo Salt Solution) to the DNA and mix by tapping the side of the tube with a finger.*

3. Transfer <600 µL of DNA/Salt solution to a GENECLEAN Turbo Cartridge assembled in a kit-supplied 2 mL capless Catch Tube.

4. Centrifuge at <14,000 x g for 5 seconds, or until all liquid has passed through the filter. Empty Catch Tube as needed.

   NOTE ▸ *If volume of DNA and GENECLEAN Turbo Salt Solution mixture is >600 µL, add the remainder of the DNA/Salt Solution to the GENECLEAN Turbo Cartridge and repeat this step until all of the liquid passes through the filter.*

   Vacuum Manifold Option: Place the end of the GENECLEAN Turbo Cartridge on a vacuum manifold. Apply vacuum to <25 inches Hg to completely drain liquid. Repeat this step until all the DNA/Salt solution has passed through the filter.

5. Add 500 µL of prepared GENECLEAN Turbo Wash to the filter.

   IMPORTANT ▸ *Be sure ethanol has been added to the GENECLEAN Turbo Wash Concentrate before using. See Section 3.1 for instructions.*

6. Centrifuge at <14,000 x g for 5 seconds. Empty the Catch Tube as needed.

   Vacuum Manifold Option: Place the GENECLEAN Turbo Cartridge on a vacuum manifold. Apply vacuum to <25 inches Hg until Cartridge is emptied of Wash. Repeat this step until all the Wash solution has passed through the filter.

   Optional: Repeat Wash procedure as detailed in Steps 5 & 6.

7. Empty the Catch Tube and centrifuge the GENECLEAN Turbo Cartridge at <14,000 x g for an additional 4 minutes to drive the last of the Wash solution from the Turbo Cartridge.

   NOTE ▸ *Centrifugation in an empty Catch Tube in this step is essential for removing trace alcohol from the final product.*

8. Remove the detachable cap from a new, clean GENECLEAN Turbo Catch Tube and set it aside. Insert the GENECLEAN Turbo Cartridge containing the bound DNA.

9. Add 30 µL GENECLEAN Turbo Elution Solution directly onto the GLASSMILK embedded membrane and incubate for 5 minutes at room temperature.

10. Centrifuge at <14,000 x g for 1 minute to transfer eluted DNA to GENECLEAN Turbo Catch Tube. Discard GENECLEAN Turbo Cartridge and cap the Catch Tube. DNA is now ready to use without further manipulation.

    NOTE ▸ *A second elution is not necessary or recommended. Repetition of this step will cause the total volume to increase and the concentration of DNA to decrease.*

5.2 Rapid Isolation of DNA from Agarose Gels

1. Place gel slice in 1.5 mL microcentrifuge tube.

   NOTE ▸ *If purifying more than 10 µg of DNA, divide sample into multiple preps.*

2. Add 100 µL of GENECLEAN Turbo Salt Solution per 0.1 g of gel slice.

   NOTE ▸ *If purifying genomic DNA, add 5 volumes of GENECLEAN Turbo GNomic Salt Solution (instead of GENECLEAN Turbo Salt Solution) to the DNA and mix by tapping the side of the tube with a finger.*

3. Incubate at 55 °C in a water bath or heat block for 5 minutes to melt gel. Invert tube to mix until the solution is homogeneous.

4. Transfer <600 µL of DNA/Salt solution to a GENECLEAN Turbo Cartridge assembled in a kit-supplied 2 mL cap-less Catch Tube.

5. Centrifuge at <14,000 x g for about 5 seconds, or until all liquid has passed through the filter. Empty Catch Tube as needed.

   NOTE ▸ *If volume of DNA and GENECLEAN Turbo Salt Solution mixture is >600 µL, add the remainder of the DNA/Salt Solution to the GENECLEAN Turbo Cartridge and repeat this step until all of the liquid passes through the filter.*

   Vacuum Manifold Option: Place the end of the GENECLEAN Turbo Cartridge on a vacuum manifold. Apply vacuum to <25 inches Hg to completely drain liquid. Repeat this step until all the DNA/Salt solution has passed through the filter.

6. Add 500 µL of prepared GENECLEAN Turbo Wash to the filter.

   IMPORTANT ▸ *Be sure ethanol has been added to the GENECLEAN Turbo Wash Concentrate before using. See Section 3.1 for instructions.*

7. Centrifuge at <14,000 x g for 5 seconds. Empty the Catch Tube as needed.

   Vacuum Manifold Option: Place the GENECLEAN Turbo Cartridge on a vacuum manifold. Apply vacuum to <25 inches Hg until Cartridge is emptied of Wash. Repeat this step until all the Wash solution has passed through the filter.

   Optional: Repeat Wash procedure as detailed in Steps 6 & 7.

8. Empty the Catch Tube and centrifuge the GENECLEAN Turbo Cartridge at <14,000 x g for an additional 4 minutes to drive the last of the Wash solution from the Turbo Cartridge.
   NOTE ▸ *Centrifugation in an empty Catch Tube in this step is essential for removing trace alcohol from the final product.*

9. Remove the detachable cap from a new, clean GENECLEAN Turbo Catch Tube and set it aside. Insert the GENECLEAN Turbo Cartridge containing the bound DNA.

10. Add 30 µL GENECLEAN Turbo Elution Solution directly onto the GLASSMILK embedded membrane and incubate for 5 minutes at room temperature.

11. Centrifuge at <14,000 x g for 1 minute to transfer eluted DNA to GENECLEAN Turbo Catch Tube. Discard GENECLEAN Turbo Cartridge and cap the Catch Tube. DNA is now ready to use without further manipulation.
    NOTE ▸ *A second elution is not necessary or recommended. Repetition of this step will cause the total volume to increase and the concentration of DNA to decrease.*

6. COMMON QUESTIONS

6.1 Does GENECLEAN Turbo work on all conformations of plasmid DNA?

Our line of Plasmid Purification Kits would be best-suited for purification of plasmid DNA. See Section 10 for related products.

6.2 Can I substitute GENECLEAN Turbo Wash Concentrate for other GENECLEAN Solutions?

No. The wash solutions have different salt concentrations and are prepared differently.

6.3 If I'm using GENECLEAN Turbo, what do I do if I have more than 10 µg of DNA or a large gel slice?

Our line of slurry-based purification kits would be best-suited for larger quantities of DNA. See Section 10 for related products.

7. TROUBLESHOOTING

7.1 Low or No Recovery with the GENECLEAN Turbo Kit

7.1.1 Problems with Binding

Yields can be affected by insufficient incubation and mixing time during the DNA/ Salt solution binding step. Inversion of the tube to mix the solution to homogeneity before loading the GENECLEAN Turbo Cartridge will increase efficiency. If purifying DNA from agarose, ensure complete melting of the gel.

7.1.2 Problems with Washing

DNA may elute in the Wash if ethanol was not added to the GENECLEAN Turbo Wash Concentrate prior to use. Prepare GENECLEAN Turbo Wash as described in Section 3.1. If the GENECLEAN Turbo Wash ethanol concentration drops significantly due to evaporation, the DNA may elute in the Wash. Store the prepared GENECLEAN Turbo Wash tightly capped at 15-30 °C.

7.1.3 Problems with Eluting

Add GENECLEAN Turbo Elution Solution directly onto the GLASSMILK embedded membrane and incubate 5 minutes at room temperature before recovering the DNA.

7.1.4 Rapid Kit Reagent Test Procedure

If yields are less than 50%, this test takes 15-20 minutes to determine if the problem is due to reagents or to some other aspect of the procedure.

1. Add 0.5–1 µg of DNA into a final volume of 20 µL H_2O or TE buffer. Transfer 10 µL into a microcentrifuge tube.
2. Add 50 µL GENECLEAN Turbo Salt Solution to the DNA in the microcentrifuge tube and mix well.

3. Transfer solution to a GENECLEAN Turbo Cartridge assembled into a 2 mL capless microcentrifuge tube. Centrifuge at <14,000 x g until all liquid has transferred to the Catch Tube. Transfer the flow-through to a new microcentrifuge tube.

4. Precipitate any DNA present in the flow-through by adding 50 µL water and 100 µL isopropanol and mix. Centrifuge at <14,000 x g for 5 minutes. Drain the tube and add 10 µL of water.

5. Add 300 µL GENECLEAN Turbo Wash and centrifuge. Save the Wash.

6. Centrifuge to dry for 4 minutes and transfer the GENECLEAN Turbo Cartridge to a new GENECLEAN Turbo Catch Tube.

7. Add 30 µL TE or H_2O directly onto the GLASSMILK embedded membrane and incubate for 5 minutes at room temperature. Centrifuge at <14,000 x g for 1 minute and save supernatant. Transfer filter to a new Catch Tube.

8. Repeat elution (step 7).

9. Run the following samples on a 0.8% agarose mini-gel until the samples migrate 1–2 cm.

Lane 2: 15 µL first elution (step 7).
Lane 3: 15 µL second elution (step 8).
Lane 4: 10 µL GENECLEAN Turbo Wash (step 5, add Ficoll®, sucrose, or glycerol to keep the GENECLEAN Turbo Wash in the well).
Lane 5: 10 µL of the precipitated GENECELAN Turbo supernatant after absorption (step 4).

The results of the gel should show the fate of DNA during the GENECLEAN Turbo procedure. Most of the DNA should be in the first elution (lane 2), some (approximately 10%) should be in the second elution (lane 3), and none in the GENECLEAN Turbo Wash or GENECLEAN Turbo Salt Solution after absorption (lanes 4 and 5, respectively). If DNA is seen in lane 5, this indicates that not all the DNA bound to the Turbo Filter. DNA in lane 4 indicates loss during the Wash step. The relative quantities of DNA in each elution will indicate efficiencies during this step. The results of this rapid kit test normally show a recovery of 70% or more in the first elution.

7.1.5 Rapid Kit Reagent Test Procedure

DNA yield can be quantified with a fluorometer or estimated by running the sample against a known amount of DNA on an agarose gel. Using a spectrophotometer to quantify DNA yield is not recommended for the following reasons:

1. Residual silica particles (which do not interfere with downstream reactions or uses of the DNA) can scatter UV light, affecting OD_{260} readings and OD_{260}/OD_{280} ratios.
2. After diluting part of your sample up to the minimum volume of the cuvette, the DNA will often be too dilute to give a significant reading. For example, if you eluted 0.5 µg in 20 µL of water and diluted 2 µL of this to 200 µL, the final concentration of DNA in the cuvette would be 0.5 µg/0.02 mL x 0.01=0.25 µg/mL. This would give an absorbance of only 0.005, which is too low to be significant on most instruments.

7.2 Multiple Bands Observed after the Purification Process

Because the melting temperature of dsDNA decreases in high salt concentrations, AT-rich fragments may denature during the gel melting step. This may reveal itself by multiple bands in a gel after removal of a single band. Single-stranded species can be renatured to dsDNA by heating and slow cooling. Alternatively, the gel melting step can be done at lower temperatures than 55 °C by rotating the tube on a turn wheel at room temperature for 15 minutes or until the agarose slice is completely dissolved.

7.3 Removing Primer Dimers (~50 bp) from Desired Fragment of 100 bp or More

To prevent the binding of primer dimers approximately 50 bp in size, use diluted GENECLEAN Turbo Wash Solution during the wash steps in the regular protocols (Section 5). To prepare diluted wash add 220 µL H_2O per 780 µL prepared GENECLEAN Turbo Wash Solution (see Section 3.1). Diluted wash should be used only when necessary as it may reduce recovery of the desired fragment.

7.4 Replacing GENECLEAN Turbo Wash Solution

The amount of GENECLEAN Turbo Wash Concentrate provided is sufficient when the protocol is followed as recommended. If the protocol is changed such that more prepared GENECLEAN Turbo Wash Solution is required, a solution of 80% ethanol can be made using TE (10 mM Tris and 1 mM EDTA, pH 7.5) and will work nearly as well as the proprietary GENECLEAN Turbo Wash Solution.

8. REFERENCES

Many of the principles of the GENECLEAN procedures described here are based on the data of Vogelstein and Gillespie (1). See reference 2 for discussion of the effects of chaotropic salts and temperature on DNA stability.

1. Vogelstein, B; Gillespie, D. *Proc. Nat. Acad. Sci. USA.* **1979**, *76*, 615.

2. Hamaguchi, K; Geiduschek, E.P. *J. Amer. Chem. Soc.* **1962**, *84*, 1329.

9. RECOMMENDED REFERENCE FORMAT

DNA was purified from gel or solution using the GENECLEAN Turbo Kit (MP Bio, Solon, Ohio).

10. RELATED PRODUCTS

10.1 Gel Isolation and Reaction Cleanup Products

Product Name	Size	Cat. No.
GENECLEAN® Turbo Kit	50 preps	111102200
	100 preps	111102400
GENECLEAN® Turbo for PCR Kit	50 preps	111103200
	100 preps	111103400
GENECLEAN® Kit	200 preps	111001200
GENECLEAN® II Kit	300 preps	111001400
GENECLEAN® III Kit	600 preps	111001600
GENECLEAN® SPIN KIT	50 preps	111101200
	100 preps	111101400
MERmaid® SPIN Kit	25 preps	111105200
	150 preps	111105600
RNaid® Kit	200 preps	111007200
RNaid® SPIN Kit	200 preps	111107200
EtBr GREENBAG™ Disposal Kit	50 bags	112350200
SPIN Module (Includes #2080-601)	60 F/T	112080600
SPIN Module (Includes #2080-801)	100 F/T	112080800
Label Block	1 mL	111001605

10.2 GENECLEAN-Based Genomic DNA Isolation Kits

Product Name	Size	Cat. No.
FastDNA™ Kit	100 preps	116540400
FastDNA™ Kit for Soil	50 preps	116560200
FastDNA™ SPIN Kit	100 preps	116540600
GNOME® DNA Isolation Kit	25 preps	112010400
	100 preps	112010600
GENECLEAN® for Ancient DNA Kit	100 preps	111002200

10.3 Plasmid Purification Products

Product Name	Size	Cat. No.
RapidPURE® Plasmid Mini Kit	60 preps	112066200
	120 preps	112066400
	300 preps	112066600
Yeast RPM® Kit	100 preps	112069400
MiniPrep Express™ Matrix	1,250 preps	112000200

11. PRODUCT USE LIMITATION & WARRANTY

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