Application Manual

GeneClean® Turbo Kit for PCR

For purification of PCR products
ranging from 100 bp to 10 kb

Cat. No. and Size:
111103200, 50 preps
111103400, 100 preps
111103600, 300 preps

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

Protocol Revision #111103200-201901

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1. Introduction

The GeneClean Turbo for PCR Kit is an advanced adaptation of the original GeneClean kit containing the patented GLASSMILK® nucleic acid binding matrix. It uses a GeneClean Turbo Cartridge system designed to further simplify the purification process. This system contains a special GLASSMILK embedded membrane and buffer system optimized for the purification of PCR products from 100 bp to 10 kb. DNA is eluted in 30 µl of H_20 or TE buffer. Turbo Cartridges also have a luer lock fitting for use with any vacuum manifold.

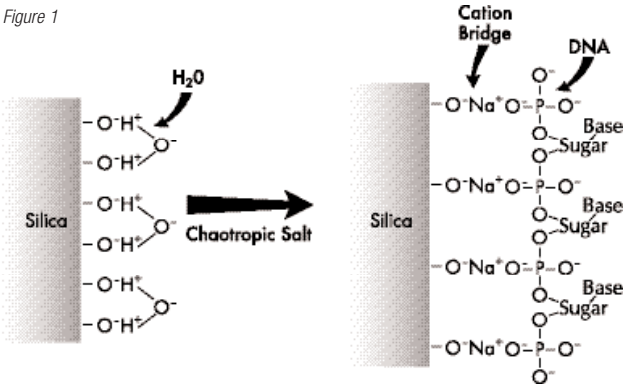
1.1 Applications for GeneClean Turbo Technology

- Desalting
- 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 in high concentrations of chaotropic salt and elutes when the salt concentration is lowered. 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 for PCR Kit Components

GeneClean Turbo for PCR Salt Solution (28 mL, Cat. No. 111103201; 55 mL, Cat. No. 111103401; 165 mL, Cat. No. 111103601) is a specially prepared aqueous solution of a guanidine chaotropic binding salt that allows the DNA to bind to the GLASSMILK embedded in the GeneClean Turbo Cartridge.

GeneClean Turbo for PCR Wash Concentrate (6 mL, Cat. No. 111103202; 11 mL, Cat. No. 111103402; 32 mL, Cat. No. 111103602) is a concentrated, proprietary salt solution to which ethanol is added to make GeneClean Turbo for PCR Wash (see Section 3.1). Store GeneClean Turbo for PCR Wash at the bench (15°–30°C). Keep tightly capped to prevent evaporation of ethanol.

GeneClean Turbo for PCR Cartridges (50, Cat. No. 111103203; 100, Cat. No. 111103403; 300, Cat. No. 111103603) contain a specially designed composite membrane that incorporates GLASSMILK as an integral part. 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 for PCR Cartridges have a luer lock fitting so they can be used in a vacuum manifold.

GeneClean Turbo for PCR Catch Tubes (50, Cat. No. 111103204; 100, Cat. No. 111103404; 300, Cat. No. 111103604) are 1.5 mL microcentrifuge tubes with removable caps. They are used to recover and store DNA eluted from the GeneClean Turbo for PCR Cartridges.

GeneClean Turbo for PCR Elution Solution (3.3 mL, Cat. No. 111103205; 6.6 mL, Cat. No. 111103405; 20 mL, Cat. No. 111103605) is RNase/DNase/pyrogen-free

water for elution of DNA from the GeneClean Turbo for PCR Cartridges.

2.2 User Supplied Materials

Benchtop microcentrifuge
200 proof (100%) ethanol
Distilled water
Vacuum manifold (optional)

3. Important Considerations Before Use

3.1 Preparation of GeneClean Turbo for PCR 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
111103200	50	6 mL	54 mL
111103400	100	11 mL	99 mL
111103600	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 for PCR 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.

4. Simplified Protocol for Experienced Users

1. Transfer 100 µL oil-free PCR reaction to a 1.5 mL microcentrifuge tube.

2. Add 5 volumes GeneClean Turbo for PCR Salt Solution and mix.
3. Transfer PCR/Salt Solution to a GeneClean Turbo for PCR Cartridge.
4. Centrifuge for 5 seconds. Empty Catch Tube as needed.
5. Add 500 µL prepared GeneClean Turbo for PCR Wash to the filter.
6. Centrifuge for 5 seconds. Empty Catch Tube as needed.
7. Empty Catch Tube. Centrifuge the GeneClean Turbo for PCR Cartridge for an additional 4 minutes to remove residual Wash Solution.
8. Remove cap from Catch Tube, insert GeneClean Turbo for PCR Cartridge containing bound DNA.
9. Add 30 µL GeneClean Turbo for PCR Elution Solution directly onto GLASSMILK-embedded membrane and incubate at room temperature for 5 minutes.
10. Centrifuge for 30 seconds to transfer eluted DNA to GeneClean Turbo for PCR Catch Tube. Discard GeneClean Turbo for PCR Cartridge and cap the Catch Tube.

5. Detailed Protocol

1. Transfer 100 µL oil-free PCR reaction to a 1.5 mL microcentrifuge tube. Spot the PCR sample onto parafilm and transfer the aqueous part 2-3 times to a new location on the parafilm, leaving the oil behind. At the last transfer, only the aqueous phase should be left.
2. Add 5 volumes GeneClean Turbo for PCR Salt Solution and mix by tapping side of tube with a finger.
3. Transfer PCR reaction/Salt Solution mixture to a GeneClean Turbo for PCR Cartridge assembled in a kit-supplied 2 mL cap-less Catch Tube.
4. Centrifuge at <14,000 x g for 5 seconds. Empty Catch Tube as needed.
   Vacuum Manifold Option: Place the GeneClean Turbo for PCR Cartridge on a vacuum manifold.
   Apply vacuum to <25 inches Hg to completely drain liquid.
5. Add 500 µL of prepared GeneClean Turbo for PCR Wash to the filter.*

***VERY IMPORTANT:** Be sure ethanol has been added to the GeneClean Turbo for PCR 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 for PCR 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 for PCR

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 GeneClean Turbo for PCR Catch Tube and set it aside. Insert the GeneClean Turbo for PCR Cartridge containing the bound DNA.
9. Add 30 µL GeneClean Turbo for PCR Elution Solution directly onto the GLASSMILK-embedded membrane and incubate for 5 minutes at room temperature.
10. Centrifuge at <14,000 x g for 30 seconds to transfer eluted DNA to GeneClean Turbo for PCR Catch Tube. Discard GeneClean Turbo for PCR 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 for PCR Wash Concentrate for other GeneClean Solutions?

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

6.3 If I am using GeneClean Turbo for PCR, what do I do if I have more than 10 µg of DNA?

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 for PCR 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 for PCR Cartridge will increase efficiency.

7.1.2 Problems with Washing

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

7.1.3 Problems with Eluting

Add GeneClean Turbo for PCR 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. Put 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 for PCR Salt Solution to the DNA in the microcentrifuge tube and mix well.
3. Transfer solution to a GeneClean Turbo for PCR Cartridge assembled into a 2 mL cap-less microcentrifuge tube. Centrifuge at <14,000 x g until all liquid has transferred to Catch Tube. Transfer the flowthrough 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 for PCR Wash and centrifuge. Save the Wash.
6. Centrifuge to dry for 2 minutes and transfer the GeneClean Turbo for PCR Cartridge to a new GeneClean Turbo for PCR Catch Tube.
7. Add 30 µL TE or H_2O Solution 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). Save this supernatant to a new tube.
9. Run the following samples on a 0.8% agarose mini-gel until the samples migrate 1–2 cm.

Lane 1: 5 µL DNA from the 10 µL left in step 1 that was not purified with the GeneClean Turbo for PCR.
Lane 2: 15 µl first elution (step 7).
Lane 3: 15 µl second elution (step 8).
Lane 4: 10 µl GeneClean Turbo for PCR Wash (step 5, add Ficoll®, sucrose, or glycerol to keep the GeneClean Turbo for PCR Wash in the well).
Lane 5: 10 µl of the precipitated GeneClean Turbo for PCR supernatant after absorption (step 4).

The results of the gel should show the fate of DNA during the GeneClean Turbo for PCR 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 GeneClean Turbo for PCR Wash or guanidine thiocyanate 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 Problems Measuring Yield

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

To prevent the binding of primer dimers approximately 50bp in size, use diluted GeneClean Turbo for PCR Wash Solution. To prepare diluted wash add 220 µL

H_2O per 780 µL prepared GeneClean Turbo for PCR Wash Solution (see Section 3.1). Diluted wash should be used only when necessary as it may reduce recovery of the desired fragment.

7.3 Replacing GeneClean Turbo for PCR Wash Solution

The amount of GeneClean Turbo for PCR Wash Concentrate provided is sufficient when the protocol is followed as recommended. If the protocol is changed such that more prepared GeneClean Turbo for PCR 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. and Gillespie, D. (1979) Proc. Nat. Acad.Sci., USA. 76, 615.
2. Hamaguchi, K. and Geiduschek, E.P. (1962) J. Amer. Chem. Soc. 84, 1329.

9. Recommended Reference Format

DNA of _____ kb was purified from solution using the GeneClean Turbo for PCR Kit (MP Biomedicals, LLC., Solon, Ohio).

10. Related Products

10.1 Gel Isolation and Reaction Cleanup Products

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

10.2 GeneClean-Based Genomic DNA Isolation Kits

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

10.3 Plasmid Purification Products

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

11. Product Use Limitation & Warranty

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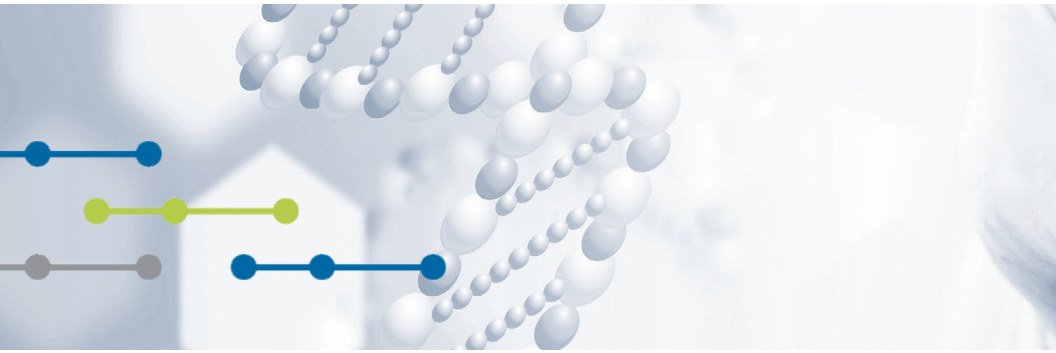
Application Manual

GeneClean Turbo for PCR Kit

Cat. No. 111103200, 111103400, 1111032600

Protocol Revision # 111103200-201901



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