1. MEDIUM FOR THE GROWTH OF *Nitrosomonas europaea,*

1.1. Medium by ATCC:

ATCC

Product Information Sheet for ATCC® 19718™

COLLECTION OF BACTERIA

ATCC NUMBER: 19718™

ORGANISM: *Nitrosomonas europaea*
Genome sequencing strain. Produces hydroxylamine oxidase [hydroxylamine oxidoreductase]. **Purified genomic DNA of this strain is available as ATCC® 19718D™.**

CITATION OF STRAIN:
If use of this strain results in a scientific publication it should be cited in that manuscript in the following manner: *Nitrosomonas europaea* ATCC® 19718™.

ATCC MEDIUM: compuestos son todos de ref. ma hidratada (comercial)
#2265 Broth: Nitrosomonas europaea Medium

Solution 1:

$(NH_4)_2SO_4$ (for 50 mM NH_4+), 4.95 g
KH_2PO_4, 0.62 g
$MgSO_4$, 0.27 g
$CaCl_2$, 0.04 g
$FeSO_4$ (30 mM in 50 mM EDTA at pH 7.0), 0.5 ml
$CuSO_4$, 0.0002 g
Distilled water, 1.2 L
Filter-sterilize

Solution 2:

KH_2PO_4, 8.2 g
NaH_2PO_4, 0.7 g
Distilled water, 300.0 ml
Bring to pH 8.0 with 10 N NaOH. Filter-sterilize

Solution 3 (buffer):

Na_2CO_3 anhydrous, 0.6 g
Distilled water, 12.0 ml
Filter-sterilize

Combine Solutions 1 and 2. Add solution 3 and filter-sterilize. Aseptically dispense into desired aliquots.

CONDITIONS:
Temperature: 26°C
Atmosphere: Aerobic

BIOSAFETY LEVEL: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

ATCC WARRANTY:
The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

DISCLAIMERS:
This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.
Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

PROPAGATION PROCEDURE:
1. Thaw frozen vial at room temperature and aseptically transfer vial contents to 10 ml of ATCC Broth #2265 contained in an Erlenmeyer flask or a tissue culture flask.
2. Incubate flask in the dark at 26°C. When using a tissue culture flask, flask should be laid down for incubation to promote air exchange.
3. Growth should be evident in a static culture in 7 to 10 days. After growth has been established the culture can be transferred (*see below*).

NOTES:
Transfer culture every four to six weeks, storing the fully-grown culture at 4°C. A 10% inoculum (10 ml per 100 ml fresh medium) is recommended. Cells may be preserved by freezing with a suitable cryoprotectant. This strain is very sensitive to impurities on glassware.
Additional information on this culture is available on the ATCC web site at www.atcc.org.

REFERENCES:
1. Sayavedra-Soto LA, Hommes NG, Arp DJ.

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

ATCC® Product Information Sheet for ATCC® 19718™

Characterization of the gene encoding hydroxylamine oxidoreductase in *Nitrosomonas europaea*. J. Bacteriol. 176:504-510, 1994. PubMed: 8288544

2. Fujiwara T, Yamanaka T, Fukumori Y. The amino acid sequence of *Nitrosomonas europaea* cytochrome c-552. Curr. Microbiol. 31:1-4, 1995. PubMed: 7767224
3. Kester RA, De Boer W, Laanbroer HJ. Production of NO and N_2O by pure cultures of nitrifying and denitrifying bacteria during changes in aeration. Appl. Environ. Microbiol. 63:3872-3877, 1997.
4. Hovanec TA, DeLong EF. Comparative analysis of nitrifying bacteria associated with freshwater and marine aquaria. Appl. Environ. Microbiol. 62:2888-2896, 1996. PubMed: 8702281

Updated (10/05) PK/SAL

1.2. Alternative medium

Improved methods for the cultivation of the chemolithoautotrophic bacterium *Nitrosomonas europaea*, B.D. Chapman, M. Schleicher, A. Beuger, P. Gostomski, J.H. Thiele, Journal of Microbiological Methods 65 (2006) 96-106.

Composition:

- 25 mM $(NH_4)_2\ SO_4$
- 0.272 mM Mg SO_4·7H2O (246.4 g)
- 0.6 mM $CaCl_2$
- 0.24 µM Fe SO_4·$7H_2O$ (278.02 g)
- 0.174 µM de EDTA·H_2O (372,24 g)
- 3 mM K_2HPO_4
- 1.4 µM phenol red

Procedure:

- pH adjustment to 7.8 with Na_2CO_3 0.47M.
- Sterilize two sealed bottles with rubber stoppers.
- 30 mL Ernlenmeyer batch culture protected from light.
- pH adjustment to 7.5-8 following the color change with sterile solutions NH_4HCO_3 1M.

2. Wizard Genomic DNA Purification Kit (Promega):

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Wizard Genomic DNA Purification Kit

III.G. Isolating Genomic DNA from Gram Positive and Gram Negative Bacteria

Materials to Be Supplied by the User

- 1.5ml microcentrifuge tubes
- water bath, 80°C
- water bath, 37°C
- isopropanol, room temperature
- 70% ethanol, room temperature
- water bath, 65°C (optional; for rapid DNA rehydration)
- 50mM EDTA (pH 8.0) (for gram positive bacteria)
- 10mg/ml lysozyme (Sigma Cat.# L7651) (for gram positive bacteria)
- 10mg/ml lysostaphin (Sigma Cat.# L7386) (for gram positive bacteria)

Gram ⊖ Gram ⊕

1. Add 1ml of an overnight culture to a 1.5ml microcentrifuge tube.
2. Centrifuge at 13,000–16,000 × *g* for 2 minutes to pellet the cells. Remove the supernatant. For Gram Positive Bacteria, proceed to Step 3. **For Gram Negative Bacteria go directly to Step 6.**
3. Resuspend the cells thoroughly in 480µl of 50mM EDTA.
4. Add the appropriate lytic enzyme(s) to the resuspended cell pellet in a total volume of 120µl (see note in the margin), and gently pipet to mix. The purpose of this pretreatment is to weaken the cell wall so that efficient cell lysis can take place.

   Note: For certain *Staphylococcus* species, a mixture of 60µl of 10mg/ml lysozyme and 60µl of 10mg/ml lysostaphin is required for efficient lysis. However, many Gram Positive Bacterial Strains (e.g., *Bacillus subtilis, Micrococcus luteus, Nocardia otitidiscaviarum, Rhodococcus rhodochrous,* and *Brevibacterium albidium*) lyse efficiently using lysozyme alone.

5. Incubate the sample at 37°C for 30–60 minutes. Centrifuge for 2 minutes at 13,000–16,000 × *g* and remove the supernatant.

Gram ⊖

6. Add 600µl of Nuclei Lysis Solution. Gently pipet until the cells are resuspended.
7. Incubate at 80°C for 5 minutes to lyse the cells; then cool to room temperature.
8. Add 3µl of RNase Solution to the cell lysate. Invert the tube 2-5 times to mix.
9. Incubate at 37°C for 15–60 minutes. Cool the sample to room temperature.
10. Add 200µl of Protein Precipitation Solution to the RNase-treated cell lysate. Vortex vigorously at high speed for 20 seconds to mix the Protein Precipitation Solution with the cell lysate.
11. Incubate the sample on ice for 5 minutes.
12. Centrifuge at 13,000–16,000 × *g* for 3 minutes.

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13. Transfer the supernatant containing the DNA to a clean 1.5ml microcentrifuge tube containing 600µl of room temperature isopropanol.

    Note: Some supernatant may remain in the original tube containing the protein pellet. Leave this residual liquid in the tube to avoid contaminating the DNA solution with the precipitated protein.

14. Gently mix by inversion until the thread-like strands of DNA form a visible mass.

15. Centrifuge at 13,000–16,000 × *g* for 2 minutes.

16. Carefully pour off the supernatant and drain the tube on clean absorbent paper. Add 600µl of room temperature 70% ethanol and gently invert the tube several times to wash the DNA pellet.

17. Centrifuge at 13,000–16,000 × *g* for 2 minutes. Carefully aspirate the ethanol.

18. Drain the tube on clean absorbent paper and allow the pellet to air-dry for 10–15 minutes.

19. Add 100µl of DNA Rehydration Solution to the tube and rehydrate the DNA by incubating at 65°C for 1 hour. Periodically mix the solution by gently tapping the tube. Alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or at 4°C.

20. Store the DNA at 2–8°C.

IV. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Comments
Blood clots present in blood samples	The tube may have been stored improperly; the blood was not thoroughly mixed, or inappropriate tubes were used for drawing blood. Discard the clotted blood and draw new samples using EDTA-, heparin- or citrate-treated anticoagulant tubes.
Poor DNA yield	The blood sample may contain too few white blood cells. Draw new blood samples.
	The white blood cell pellet was not resuspended thoroughly in Step 5 of Section III.A or B. The white blood cell pellet must be vortexed vigorously to resuspend the cells.
	The blood sample was too old. Best yields are obtained with fresh blood. Samples that have been stored at 2–5°C for more than 5 days may give reduced yields.

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3. AGAROSE GEL ELECTROPHORESIS:

- To do a 2% agarose gel: weight 1g of agarose.
- To do a 1% agorse gel: weight 0.5g of agarose.
- Dissolve it in 50 ml of 1xTAE in an Erlenmeyer (microwave, 30s-1min until it becomes transparent.
- While it cools down, prepare a cuvette.
- Add 25µL of ethidium bromide (carcinogen)
- Let the gel cool down 20 min.
- Load the sample.
- To do checking gels: 1 µL of buffer+1-2 µL of sample.
- To purify DNA: all the sample + buffer 6x.

4. Wizard SV Gel and PCR Clean- Up System (Promega):

Wizard® SV Gel and PCR Clean-Up System

INSTRUCTIONS FOR USE OF PRODUCTS A9280, A9281, AND A9282

PROTOCOL

Gel Slice and PCR Product Preparation

A. Dissolving the Gel Slice

1. Following electrophoresis, excise DNA band from gel and place gel slice in a 1.5ml microcentrifuge tube.
2. Add 10µl Membrane Binding Solution per 10mg of gel slice. Vortex and incubate at 50–65°C until gel slice is completely dissolved.

B. Processing PCR Reactions

1. Add an equal volume of Membrane Binding Solution to the PCR reaction.

Binding of DNA

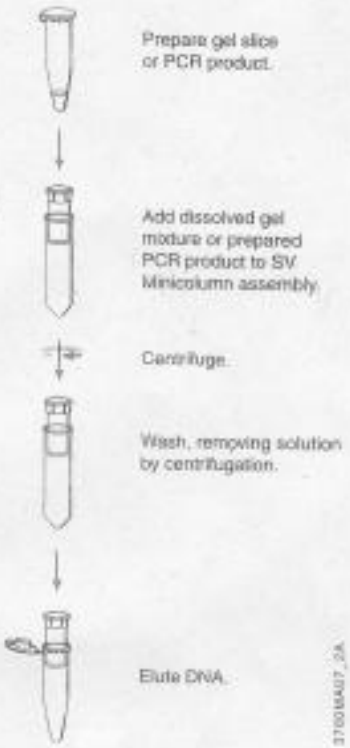
1. Insert SV Minicolumn into Collection Tube.
2. Transfer dissolved gel mixture or prepared PCR product to the Minicolumn assembly. Incubate at room temperature for 1 minute.
3. Centrifuge at 16,000 × *g* for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.

Washing

4. Add 700µl Membrane Wash Solution (ethanol added). Centrifuge at 16,000 × *g* for 1 minute. Discard flowthrough and reinsert Minicolumn into Collection Tube.
5. Repeat Step 4 with 500µl Membrane Wash Solution. Centrifuge at 16,000 × *g* for 5 minutes.
6. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.

Elution

7. Carefully transfer Minicolumn to a clean 1.5ml microcentrifuge tube.
8. Add 50µl of Nuclease-Free Water to the Minicolumn. Incubate at room temperature for 1 minute. Centrifuge at 16,000 × *g* for 1 minute.
9. Discard Minicolumn and store DNA at 4°C or –20°C.



Additional protocol information is available in Technical Bulletin #TB308, available upon request from Promega or online at www.promega.com

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5. GoTaq Green Master Mix (Promega):

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Usage Information

I. Standard Application

Reagents to be Supplied by the User

template DNA — downstream primer
upstream primer — mineral oil (optional)

1. Thaw the GoTaq® Green Master Mix at room temperature. Vortex the Master Mix, then spin it briefly in a microcentrifuge to collect the material at the bottom of the tube.
2. Prepare one of the following reaction mixes on ice:

For a 25µl reaction volume:

Component	Volume	Final Conc.
GoTaq® Green Master Mix, 2X	12.5µl	1X
upstream primer, 10µM	0.25–2.5µl	0.1–1.0µM
downstream primer, 10µM	0.25–2.5µl	0.1–1.0µM
DNA template	1–5µl	<250ng (100 ng)
Nuclease-Free Water to	25µl	N.A.

For a 50µl reaction volume:

Component	Volume	Final Conc.
GoTaq® Green Master Mix, 2X	25µl	1X
upstream primer, 10µM	0.5–5.0µl	0.1–1.0µM
downstream primer, 10µM	0.5–5.0µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	50µl	N.A.

For a 100µl reaction volume:

Component	Volume	Final Conc.
GoTaq® Green Master Mix, 2X	50µl	1X
upstream primer, 10µM	1.0–10.0µl	0.1–1.0µM
downstream primer, 10µM	1.0–10.0µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	100µl	N.A.

3. If using a thermal cycler without a heated lid, overlay the reaction mix with 1–2 drops (approximately 50µl) of mineral oil to prevent evaporation during thermal cycling. Centrifuge the reactions in a microcentrifuge for 5 seconds.
4. Place the reactions in a thermal cycler that has been preheated to 95°C. Perform PCR using your standard parameters.

II. General Guidelines for Amplification by PCR

A. Denaturation

- Generally, a 2-minute initial denaturation step at 95°C is sufficient.
- Subsequent denaturation steps will be between 30 seconds and 1 minute.

B. Annealing

- Optimize the annealing conditions by performing the reaction starting approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 1°C to the annealing temperature.
- The annealing step is typically 30 seconds to 1 minute.

C. Extension

- The extension reaction is typically performed at the optimal temperature for *Taq* DNA polymerase, which is 72–74°C.
- Allow approximately 1 minute for every 1kb of DNA to be amplified.
- A final extension of 5 minutes at 72–74°C is recommended.

D. Refrigeration

- If the thermal cycler has a refrigeration or "soak" cycle, the cycling reaction can be programmed to end by holding the tubes at 4°C for several hours.
- This cycle can minimize any polymerase activity that might occur at higher temperatures, although this is not usually a problem.

E. Cycle Number

- Generally, 25–30 cycles result in optimal amplification of desired products.
- Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.

III. General Considerations

A. GoTaq® Green Master Mix Compatibility

GoTaq® Green Master Mix is compatible with common PCR additives such as DMSO and betaine. These additives neither change the color of GoTaq® Green Master Mix nor affect dye migration.

If both agarose gel analysis and further downstream applications involving absorbance or fluorescence will be used, the two dyes can be removed from reactions using standard PCR clean-up systems such as the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281).

B. Primer Design

PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Primers should contain 40–60% (G + C), and care should be taken to avoid sequences that might produce internal secondary structure. The 3'-ends of the primers should not be complementary to avoid the production of primer-dimers. Primer-dimers unnecessarily deplete primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3'-end of the primer, as this may result in nonspecific primer annealing, increasing the synthesis of undesirable reaction products. Ideally, both primers should have nearly identical melting temperatures (T_m); in this manner, the two primers should anneal roughly at the same temperature. The annealing temperature of the reaction is dependent upon the primer with the lowest T_m. For assistance with calculating the T_m of any primer, a T_m Calculator is provided on the BioMath page of the Promega web site at: **www.promega.com/biomath/**

C. Amplification Troubleshooting

To overcome low yield or no yield in amplifications (e.g., mouse tail genotyping applications), we recommend the following suggestions:

- Adjust annealing temperature. The reaction buffer composition affects the melting properties of DNA. See BioMath Calculator to calculate the melting temperature for primers in the GoTaq® reaction (**www.promega.com/biomath/**).
- Minimize the effect of amplification inhibitors. Some DNA isolation procedures, particularly genomic DNA isolation, can result in the copurification of amplification inhibitors. Reduce the volume of template DNA in reaction or dilute template DNA prior to adding to reaction. Diluting samples even 1:10,000 has been shown to be effective in improving results, depending on initial DNA concentration.
- Increase template DNA purity. Include an ethanol precipitation and wash step prior to amplification to remove inhibitors that copurify with the DNA.
- Add PCR additives. Adding PCR-enhancing agents (e.g., DMSO or betaine) may improve yields. General stabilizing agents such as BSA (Sigma Cat.# A7030; final concentration 0.16mg/ml) also may help to overcome amplification failure.

D. More Information on Amplification

More information on amplification is available online at the Promega web site:
PCR Applications: **www.promega.com/paguide/chap1.htm**
PCR Protocols and Reference: **www.promega.com/guides/pcr_guide/default.htm**

Part# 9PIM711
Printed in USA. Revised 3/08

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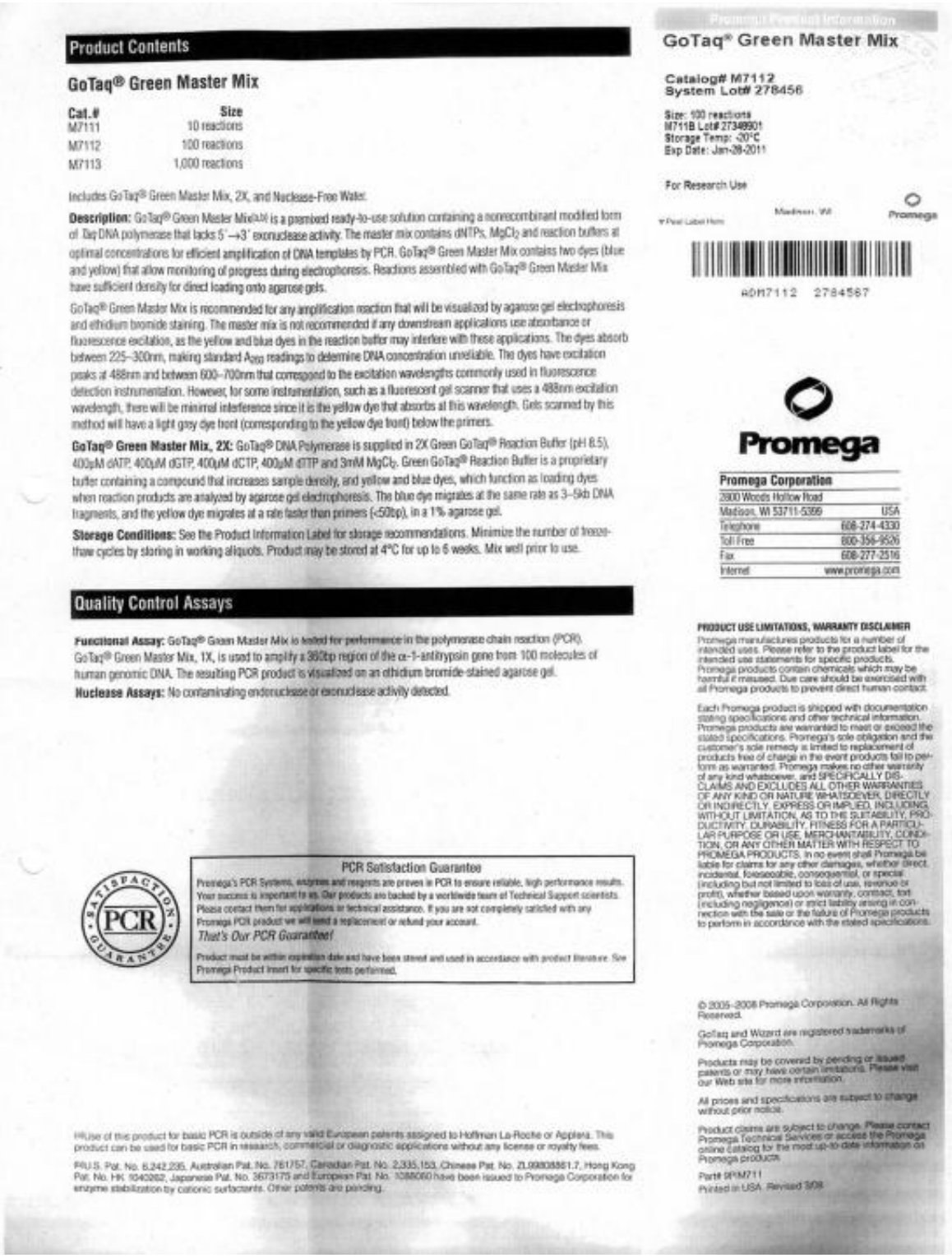

Product Contents

GoTaq® Green Master Mix

Cat.#	Size
M7111	10 reactions
M7112	100 reactions
M7113	1,000 reactions

Includes GoTaq® Green Master Mix, 2X, and Nuclease-Free Water.

Description: GoTaq® Green Master Mix[AM] is a premixed ready-to-use solution containing a nonrecombinant modified form of Taq DNA polymerase that lacks 5'→3' exonuclease activity. The master mix contains dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. GoTaq® Green Master Mix contains two dyes (blue and yellow) that allow monitoring of progress during electrophoresis. Reactions assembled with GoTaq® Green Master Mix have sufficient density for direct loading onto agarose gels.

GoTaq® Green Master Mix is recommended for any amplification reaction that will be visualized by agarose gel electrophoresis and ethidium bromide staining. The master mix is not recommended if any downstream applications use absorbance or fluorescence excitation, as the yellow and blue dyes in the reaction buffer may interfere with these applications. The dyes absorb between 225–300nm, making standard A_{260} readings to determine DNA concentration unreliable. The dyes have excitation peaks at 488nm and between 600–700nm that correspond to the excitation wavelengths commonly used in fluorescence detection instrumentation. However, for some instrumentation, such as a fluorescent gel scanner that uses a 488nm excitation wavelength, there will be minimal interference since it is the yellow dye that absorbs at this wavelength. Gels scanned by this method will have a light gray dye front (corresponding to the yellow dye front) below the primers.

GoTaq® Green Master Mix, 2X: GoTaq® DNA Polymerase is supplied in 2X Green GoTaq® Reaction Buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM $MgCl_2$. Green GoTaq® Reaction Buffer is a proprietary buffer containing a compound that increases sample density, and yellow and blue dyes, which function as loading dyes when reaction products are analyzed by agarose gel electrophoresis. The blue dye migrates at the same rate as 3–5kb DNA fragments, and the yellow dye migrates at a rate faster than primers (~50bp), in a 1% agarose gel.

Storage Conditions: See the Product Information Label for storage recommendations. Minimize the number of freeze-thaw cycles by storing in working aliquots. Product may be stored at 4°C for up to 6 weeks. Mix well prior to use.

Quality Control Assays

Functional Assay: GoTaq® Green Master Mix is tested for performance in the polymerase chain reaction (PCR). GoTaq® Green Master Mix, 1X, is used to amplify a 360bp region of the α-1-antitrypsin gene from 100 molecules of human genomic DNA. The resulting PCR product is visualized on an ethidium bromide-stained agarose gel.

Nuclease Assays: No contaminating endonuclease or exonuclease activity detected.

PCR Satisfaction Guarantee

Promega's PCR Systems, enzymes and reagents are proven in PCR to ensure reliable, high performance results. Your success is important to us. Our products are backed by a worldwide team of Technical Support scientists. Please contact them for applications or technical assistance. If you are not completely satisfied with any Promega PCR product we will send a replacement or refund your account.

That's Our PCR Guarantee!

Product must be within expiration date and have been stored and used in accordance with product literature. See Promega Product Insert for specific tests performed.

Premium Product Information

GoTaq® Green Master Mix

Catalog# M7112
System Lot# 278456

Size: 100 reactions
M711B Lot# 27348901
Storage Temp: -20°C
Exp Date: Jan-28-2011

For Research Use

Made in Madison, WI

Promega

ADM7112 2784587

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Nitrite PCR solution:

- GoTaq -> 12.5 µL
- Primers -> 2.5 microl each
- DNA (*Nitrosomonas*) -> 1 µL (104 ng)
- H_2O (nuclease free water) -> 6.5 µL

Phosphate PCR solution:

- GoTaq -> 12.5 µL
- Primers -> 2.5 µL each
- DNA (*E. coli*) -> 1.5 µL (132 ng)
- H_2O (nuclease free water) -> 6 µL

Thermocycler PCR cycles:

1) 5 min at 95 ºC
2) 45 s at 95 ºC
3) 1 min at the lowest hibridation temperature of the interesting primers
4) 1 min at 72 ºC
5) Repeat 30 times from step 2 (go to 2) -> 30 PCR cycles
6) 5 min at 72 ºC
7) Keep it at 4ºC

6. AGAR PLATES:

- Weight 10g of Trypone, 5g of Yeast Extract, 10g NaCl and 15g of agar.
- Add 1 L of distilled water.
- Sterilize LB+agar medium in an Erlenmeyer.
- Let chill it till 55ºC and add the antibiotic.
- Pour the medium in the plates, let it solidify, then turn it and keep it on the fridge (wrapped on foil)

7. SOC MEDIUM FOR *Escherichia coli*:

Composition:

- 25 mM $(NH_4)_2\ SO_4$
- Tryptone (pancreatic digest of casein) 2% (w/v)
- Yeast extract 0.5% (w/v)
- NaCl 8.6 mM
- KCl 2.5 mM
- $MgSO_4$ 20 mM
- Glucose 20 mM

Procedure:

- SOC media should have its pH adjusted to 7.0 by adding concentrated sodium hydroxide.
- Finally, the medium should be autoclaved at 121°C to ensure sterility.

8. PREPARATION OF COMPETENT *Escherichia coli* CELLS (chemically competent cells):

(A rapid and highly efficient method for preparation of competent Escherichia coli cells' *A. Nishimura, M.Morita, Y. Nishimura and Y. Sugino*, National Institute of Genetics, Nucleic Acids Research, Vol, 18, No 20 6169, Hirata Osaka, Japan, July 2, 1990)

- 50ml of medium A (in falcons) + 0.5 ml of E. coli ON culture.
- Let it grow until it arrives to half log phase.
- Chill it 10 min in ice.
- Centrifuge it 10 min to 15000g and 4ºC.
- Resuspend 0.5ml medium A (in ice)
- Add 20.5 ml of solution B and shake it in vortex (by inversion).
- Do some 0.1 mL aliquots and keep it at -80ºC.

Medium A → LB supplemented with 10 mM MgSO4·7H2O and 0.2% glucose

Solution B → LB with 36% glycerin, 12% PEG (MW7500) and 12 mM MgSO4·7H2O. Sterilized by filtration.

9. TRANSFORMING CHEMICALLY COMPETENT *Escherichia coli* CELLS:

- Blend between 5 µl to 10 µl of the ligation with 50 µl of competent cells (if it is 2 to 5 µl plasmid).
- Incubate for 30 minutes in ice.
- Incubate at 42ºC for 45 seconds.
- Put it 1 minute in ice.
- Add 500 µl of medium LB/SOB/SOC (they should be at room temperature). To increase the efficiency use SOC medium.
- Incubate for 60 minutes at 37ºC in a shaker. Put the plates in the heater at 37ºC. Spread 100 µl, 200 µl and the rest on LB+antibiotic plates.

10. Wizard Plus SV Minipreps DNA Purification System (Promega):

Promega

3. Protocols

Do not exchange or replace components of the Wizard® *Plus* SV Minipreps DNA Purification System with components from any other Wizard® *Plus* System. Components from the Wizard® *Plus* and Wizard® *Plus* SV Systems are not interchangeable.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 6.)

- LB agar plates containing antibiotic
- LB medium containing antibiotic
- ethanol (95%)
- microcentrifuge capable of 14,000 × *g*
- sterile 1.5ml microcentrifuge tubes
- centrifuge capable of 10,000 × *g*

Prior to using a new Wizard® *Plus* SV Minipreps DNA Purification System, dilute the provided Column Wash Solution (CWA) as follows:

Add 7ml of 95% ethanol for a final volume of 11ml for the 10-prep system (Cat.# A1270).

Add 35ml of 95% ethanol for a final volume of 55ml for the 50-prep system (Cat.# A1330 and A1340).

Add 170ml of 95% ethanol for a final volume of 270ml for the 250-prep system (Cat.# A1460 and A1470).

3.A. Preparation of *E. coli*

1. Use a single, well-isolated colony from a fresh Luria-Bertani (LB) agar plate (containing antibiotic) to inoculate 1–10ml of LB medium (containing the same antibiotic). We recommend LB culture medium. Rich media, such as Terrific Broth, produce high cell densities that may overload the DNA purification system.
2. Incubate overnight (12–16 hours) at 37°C in a shaking incubator. Incubation time can be optimized to increase the plasmid DNA yield for a given culture volume. However, it has been observed that as a culture ages the DNA yield may begin to decrease due to cell death and lysis within the culture.

Note: An A_{600} reading of 2–4 ensures that cells have reached the proper growth density for harvesting and plasmid DNA isolation.

For high-copy-number plasmids, do not process more than 5ml of bacterial culture. If more than 5ml of culture is processed, the capacity of the Wizard® SV Minicolumn will be exceeded and no increase in plasmid yield will be obtained.

For low-copy-number plasmids, it may be necessary to process larger volumes of bacterial culture (up to 10ml) for recovery of sufficient DNA. Processing greater than 10ml of culture will lead to insufficient clearing of the bacterial lysate and thus increased contaminants in the plasmid DNA.

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3.B. Production of a Cleared Lysate

Note: Throughout the remainder of this document, the supplied Cell Resuspension Solution (CRA), Cell Lysis Solution (CLA), Neutralization Solution (NSE) and Column Wash Solution (CWA) are referred to as Cell Resuspension Solution, Cell Lysis Solution, Neutralization Solution and Column Wash Solution, respectively.

1. Harvest 1–5ml (high-copy-number plasmid) or 10ml (low-copy-number plasmid) of bacterial culture by centrifugation for 5 minutes at 10,000 × *g* in a tabletop centrifuge. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media.
2. Add 250µl of Cell Resuspension Solution and completely resuspend the cell pellet by vortexing or pipetting. It is essential to thoroughly resuspend the cells. If they are not already in a microcentrifuge tube, transfer the resuspended cells to a sterile 1.5ml microcentrifuge tube(s).

Note: To prevent shearing of chromosomal DNA, do not vortex after Step 2. Mix only by inverting the tubes.

3. Add 250µl of Cell Lysis Solution and mix by inverting the tube 4 times (do not vortex). Incubate until the cell suspension clears (approximately 1–5 minutes).

   Note: It is important to observe partial clearing of the lysate before proceeding to addition of the Alkaline Protease Solution (Step 4); however, do not incubate for longer than 5 minutes.

4. Add 10µl of Alkaline Protease Solution and mix by inverting the tube 4 times. Incubate for 5 minutes at room temperature.

Alkaline protease inactivates endonucleases and other proteins released during the lysis of the bacterial cells that can adversely affect the quality of the isolated DNA. Do not exceed 5 minutes of incubation with Alkaline Protease Solution at Step 4, as nicking of the plasmid DNA may occur.

5. Add 350µl of Neutralization Solution and immediately mix by inverting the tube 4 times (do not vortex).
6. Centrifuge the bacterial lysate at maximum speed (around 14,000 × *g*) in a microcentrifuge for 10 minutes at room temperature.

3.C. Plasmid DNA Isolation and Purification Protocols

The Wizard® *Plus* SV Minipreps DNA Purification System allows a choice of methods for purification of plasmid DNA when systems with Vacuum Adapters are purchased (Cat.# A1340, A1470). Plasmid DNA may be purified from the bacterial lysate using microcentrifugation to force the cleared lysate through the Wizard® SV Minicolumn and wash the plasmid DNA. Alternatively, a vacuum can be used to pull the lysate through the Spin Column and wash the plasmid DNA. Vacuum Adapters allow the use of a vacuum manifold (e.g., a Vac-Man® Laboratory Vacuum Manifold) and vacuum source for DNA purification.

11. BIOBRICKS DESIGN

11.1. NITRITE PRIMERS:

nirk: ***E-N-X— nirk – S***

Reverse:

S
GTCAACGT ACTAGT CTGCACGAAAGACCTTTAAATAC

Temperature annealing = 62 ºC

Forward:

E N X
CAGTAGTA GAATTC GCGGCCGC TCTAGA GTTGCGCGAAATACCGTAAC

Temperature annealing= 60 ºC

nsrR*:** ***X – nsrR – S – N- P

Reverse:

X RBS Linker
GTCAACGT TCTAGA *TAAGGAGG* AATATAA **ATG**AGACTGACGAATTACAGCGATTAC

Temperature annealing = 76 ºC

Forward:

P N S
CAGTAGTA CTGCAG GCGGCCGC ACTAGT GCCAAGCTGACCTACAGTTTATTCC

Temperature annealing = 76 ºC

Oligonucleotide Data Sheet

biomers.net
the biopolymer factory

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Order No.: 00092698
Customer No.: 12523
(Please cite for inquiries)

Wednesday, September 30, 2009

nirK_rev
00092698_1 DNA

5'-gtc aac gta cta gtc tgc acg aaa gac ctt taa ata c-3'

Scale	XS	**Length**	37	**Purific.**	Cartridge
Yield	5,0 OD	**Tm**	64 °C	Getrocknet	
	11,9 nmol	**GC Content**	40,00%	**5'-Mod.:**	-
	134,6 µg	A: 13,0 C: 9,0 G: 6,0 T: 9,0		**3'-Mod:**	-
Vol.f.100pmol/µl	119 µl	**Mol.weight**		**Internal Mod.:**	
Conc.	- pmol/µl	**Calc.**	11326 g/mol	-	-
Dissolved in	- µl	**Found**	11332 g/mol	-	-

MS

nirK_forw
00092698_2 DNA

5'-cag tag tag aat tcg cgg ccg ctc tag agt tgc gcg aaa tac cgt aac -3'

Scale	XS	**Length**	48	**Purific.**	Cartridge
Yield	13,5 OD	**Tm**	79 °C	Getrocknet	
	25,3 nmol	**GC Content**	52,00%	**5'-Mod.:**	-
	374,6 µg	A: 13,0 C: 12,0 G: 13,0 T: 10,0		**3'-Mod:**	-
Vol.f.100pmol/µl	253 µl	**Mol.weight**		**Internal Mod.:**	
Conc.	- pmol/µl	**Calc.**	14803 g/mol	-	-
Dissolved in	- µl	**Found**	14821 g/mol	-	-

MS

nsrR_rev
00092698_3 DNA

5'-gtc aac gtt cta gat aag gag gaa tat aaa tga gac tga cga att aca gcg att ac-3'

Scale	XS	**Length**	56	**Purific.**	Cartridge
Yield	13,3 OD	**Tm**	73 °C	Getrocknet	
	19,8 nmol	**GC Content**	37,00%	**5'-Mod.:**	-
	343,6 µg	A: 22,0 C: 8,0 G: 13,0 T: 13,0		**3'-Mod:**	-
Vol.f.100pmol/µl	198 µl	**Mol.weight**		**Internal Mod.:**	
Conc.	- pmol/µl	**Calc.**	17377 g/mol	-	-
Dissolved in	- µl	**Found**	- g/mol	-	-

No mass spec available for technical reasons.

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narR_forw
00092698_4 DNA

5'-cag tag tac tgc agg cgg ccg cac tag tgc caa gct gac cta cag ttt att cc-3'

No mass spec available for technical reasons.

Scale	XS	**Length**	53	**Purific.**	Cartridge
Yield	11,4 OD	**Tm**	81 °C	Getrocknet	
	20,1 nmol	**GC Content**	54,00%	**5'-Mod.:**	-
	326,9 µg	A: 12,0 C: 16,0 G: 13,0 T: 12,0		**3'-Mod:**	-
Vol.f.100pmol/µl	201 µl	**Mol.weight**		**Internal Mod.:**	
Conc.	- pmol/µl	**Calc.**	16255 g/mol	-	-
Dissolved in	- µl	**Found**	- g/mol	-	-

P_rev
00092698_5 DNA

5'-tac agt ttg cgg ccg cca cct tgt cgg ggg cgg tgt -3'

MS

Scale	XS	**Length**	36	**Purific.**	Cartridge
Yield	9,2 OD	**Tm**	84 °C	Getrocknet	
	25,1 nmol	**GC Content**	66,00%	**5'-Mod.:**	-
	278,7 µg	A: 3,0 C: 10,0 G: 14,0 T: 9,0		**3'-Mod:**	-
Vol.f.100pmol/µl	251 µl	**Mol.weight**		**Internal Mod.:**	
Conc.	- pmol/µl	**Calc.**	11117 g/mol	-	-
Dissolved in	- µl	**Found**	11118 g/mol	-	-

P_forw
00092698_6 DNA

5'-cta aca tga tga att cga ctc cga cga gcg ggt gac -3'

MS

Scale	XS	**Length**	36	**Purific.**	Cartridge
Yield	14,9 OD	**Tm**	74 °C	Getrocknet	
	36,9 nmol	**GC Content**	52,00%	**5'-Mod.:**	-
	409,8 µg	A: 10,0 C: 9,0 G: 10,0 T: 7,0		**3'-Mod:**	-
Vol.f.100pmol/µl	369 µl	**Mol.weight**		**Internal Mod.:**	
Conc.	- pmol/µl	**Calc.**	11095 g/mol	-	-
Dissolved in	- µl	**Found**	11103 g/mol	-	-

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Chandra Mohan Sarkar

11.2. PHOSPHATE PRIMERS:

phoa: ***E-N-X-phoa-S***

Forward:

5' AGATTCA GAATTC GCGGCCGC TCTAGA GTTTCAACAGCTGTCATAAAGTTGTCAC 3'
E N X

Tempertature annealing: 74ºC

Reverse:

5' GCTCATA ACTAGT TTTATTTTCTCCATGTACAAATACATTAAAA 3'
S

Tempertature annealing: 74ºC