

AmpliGrid GoTaq® PCR System

This reagent kit has been especially designed for the use with the Advalytix AmpliGrid System and is only available through Advalytix.

Order Information

Ref.-No	Product	Description
OAX04524	AmpliGrid GoTaq® PCR Kit	AmpliGrid GoTaq® PCR Kit Sufficient for 5 AmpliGrid AG480F slides. No AmpliGrid slides included!

Contents

Part No	Component	Volume
DM241X	10x Gold Star Reaction Buffer	40 µL
M830X	GoTaq® DNA Polymerase, 5 Units/µL	15 µL
P119X	Nuclease Free Water	500 µL

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74°C under the following reaction conditions: 50 mM Tris-HCl (pH 9.0); 50 mM MgCl₂; 200 µM each of dATP, dGTP, dCTP, dTTP (a mix of unlabeled and [³H]dTTP); 10 µg activated calf thymus DNA; 0.1 mg/mL BSA in a final volume of 50 µL.

Storage Conditions

Store all components at -20°C.

Quality Control

Nuclease Contamination

All solutions are tested for the absence of contaminating nuclease.

Enzyme physical purity

GoTaq® DNA Polymerase is determined to be >90% pure as judged by SDS-polyacrylamide gel electrophoresis with Coomassie® Blue staining.

Customer Support

Beckman Coulter Biomedical GmbH
 Advalytix Products
 Sauerbruchstraße 50
 81377 Munich - Germany
 Telephone +49 (0) 89 579 589 3540
 Fax +49 (0) 89 579 589 3503
 E-Mail support@advalytix.com

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Manufacturer

Promega Corporation
 2800 Woods Hollow Road
 Madison, WI 53711-5399 USA



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The AmpliGrid GoTaq PCR System is for research use only. Not for use in diagnostic procedures.

Use of this product for basic PCR is outside of any valid patents assigned to Hoffman La-Roche or Applera. This product can be used for basic PCR in research or commercial without any license or royalty fees.

User Guide

The AmpliGrid GoTaq® PCR System has been optimised for the use with 1 µL amplification reactions on the AmpliGrid AG480F slide. Please refer to the AG480F user guide for further information about the AmpliGrid system before using this reagent kit.

Materials

- AmpliGrid AG480F DNA free microliter reaction slide, 48 reaction sites each, incl. sealing solution
- Upstream primer
- Downstream primer
- Template DNA/single cell
- AmpliSpeed slide cycler (Advantix AG, OAX04101 - OAX04103)

Experimental Procedure

- In a sterile, nuclease-free microcentrifuge tube, combine the following components on ice:

Table 1: Composition of master mix.

Component	Final Volume 1 AG480F	Final Conc.
10x GoldStar Buffer ¹	6 µL	1x
Upstream primer	X µL	0.1 - 0.5 µM
Downstream primer	Y µL	0.1 - 0.5 µM
GoTaq® DNA Polymerase (5 U/µL)	1.8 µL	0.15 U/µL
Template (if not predeposited on AmpliGrid slide)	Z µL	< 2 ng human genomic DNA equivalent/µL
Nuclease free water	ad 60 µL	

¹Thaw completely and vortex thoroughly prior to use.

- Mix gently by vortexing and spin down shortly.
- Put the AmpliGrid slide on a dark surface to ensure good visualisation of the engraved markings.
- Pipette 1 µL of the PCR master mix to each of the AmpliGrid reaction sites.
- Cover each droplet with 5 µL of sealing solution.
NOTE: Ensure that there is no evaporation of the master mix before covering with the sealing solution. A divided workflow might be advisable.
- Perform PCR on the AmpliSpeed slide cycler using your standard parameters.
An example profile is given in table 2.

Table 2: Recommended thermal cycling conditions for GoTaq® DNA Polymerase-mediated PCR amplification. These guidelines are optimised for the Advantix AmpliSpeed slide cycler instrument. Annealing temperature should be optimised for each primer set based on the primer T_m .

Temperature [°C]	Duration	
95	2 - 10 min	
95	30 sec	
45 - 65	60 sec	30 cycles
72	60 sec	
72	5 min	

General Considerations

Buffer Choice

The reaction buffers are compatible with common PCR additives such as DMSO and betaine.

Enzyme Concentration

Advantix has found that 0.08 - 0.15 units of GoTaq® DNA polymerase per 1 µL amplification reaction is adequate for most amplifications. Adding extra enzyme generally does not produce significant increases in yield. However, in some cases, more enzyme may be beneficial.

Single Cell Templates

Using single cells as a template for PCR is the main application for the AmpliGrid PCR system. However, some guidelines have taken into account when amplifying DNA from single cells. Please do always ensure proper cell lysis. Usually an extended initial denaturation of about 10 minutes is sufficient to lyse the cells. Some like e.g., epithelial cells from buccal swabs might show high resistance to thermal lysis. In this cases proteinase treatments may be beneficial.

Be sure to inactivate those enzymes with a heat step according to the manufacturer before adding the GoTaq® DNA Polymerase.

Polymerase concentration is another crucial point during single cell PCR. The recommended unit concentration is a good base for starting with further optimisation.