

AmpliGrid Single Cell One-Step RT-PCR System

Pre-optimized for performance and compatibility with AmpliGrid single cell and 1 µL RT-PCR.

Order Information

Ref.-No	Product
OAX04515	AmpliGrid Single Cell One-Step RT-PCR System

Contents

Part No	Component	Volume
OAX04271	2x Single Cell RT Reaction Buffer	250 µL
OAX04272	Single Cell RT Enzyme Mix	20 µL
OAX04273	5x Single Cell RT enhancer	60 µL
OAX04274	RNase Inhibitor (10 U/µL)	10 µL
OAX04276	DNase I Reaction Buffer (DNase I not included)	40 µL
OAX04275	Nuclease free water (DEPC treated)	1000 µL

Storage Conditions

Store all components at -20°C.

Shipment Conditions

Ship components on dry ice or blue ice.

Expiry

Please see kit & vial labelling for expiry dates.

Quality Control

DNA Contamination

All solutions are tested for the absence of contaminating DNA.

DNase/RNase contamination

All solutions are tested for the absence of contaminating DNase and RNase.

Kit performance

Amplification efficiency, processivity and sensitivity of the reagents are tested with an RNA dilution series.

User Guide

The AmpliGrid Single Cell One-Step RT-PCR System has been designed for highly sensitive one-step RT-PCR reactions using single cell or low copy number RNA templates. The kit contains a hot-start DNA polymerase and a highly sensitive reverse transcriptase.

The kit provides highly specific reverse transcription and PCR on the AmpliGrid slide, using gene-specific primers on either total RNA or mRNA from single cell templates. The proprietary buffer is highly optimized and balanced, leading to outstanding results.

The single cell template amount should be in the range of 1 to 50 cells. When using isolated RNA, starting amounts from 5 pg to 1ng have been tested. After cDNA synthesis has been performed, the reaction is heated to 95°C for 10 minutes to inactivate the RT enzymes, and simultaneously to activate the hot-start DNA polymerase.

Materials

- AmpliGrid DNA free microliter reaction slide
- Sealing solution (delivered with the AmpliGrid slide)
- Upstream/downstream primer mix (20 µM each)
- Template RNA / single cell templates
- AmpliSpeed slide cycler (Advantix, e.g. OAX04101)
- Optional: DNase I (e.g., Promega M610A)

Experimental Procedure

Cell Deposition

- Deposit single cells in 1x PBS on the AmpliGrid reaction sites (e.g., using FACS™, micromanipulation or laser capture microdissection)
- Note: Do not exceed a deposition volume of 100 nL as PBS will inhibit enzymatic reactions. In case of higher volumes needed for cell deposition dilute PBS (max. dilution 0.05 x PBS)

- Air dry the cells
- Optional: Perform a visual QC of cell deposition using a microscope. We strongly recommend to take advantage of this key benefit of the AmpliGrid platform as it enables you to correlate RT-PCR results with template presence or absence.
- Continue with reaction setup either with or without on-slide DNase digestion.

NOTE: DNase digestion can be skipped if using an intron spanning primer design to eliminate DNA amplicons or to distinguish between RNA and DNA amplicons.

One Step RT-PCR without DNase I digestion

NOTE: Primers for RT-PCR can either be contained in the mastermix or pre-deposited on the AmpliGrid reaction sites. Primers have to be dissolved in nuclease free water at a suggested concentration of 0.2 - 0.6 µM each. Optimization of primer concentrations might be necessary especially for multiplex systems. Let 1 µL of the primers air dry at room temperature or at 37°C before adding the master mix. Alternatively add primers to master mix as shown optional in table 1.

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

1 Table 1: Composition of master mix

Component	Volume 1 rxn	Volume 48 rxn
2x Single Cell RT Reaction Buffer	0.50 µL	30.00 µL
RNase Inhibitor (10 U/µL)	0.02 µL	1.20 µL
5x Single Cell RT Enhancer	0.15 µL	9.0 µL
Optional: Primer mix (20 µM each)	0.01 - 0.03 µL	1.2 - 1.8 µL
Single Cell RT Enzyme Mix	0.04 µL	2.40 µL
Optional: total RNA control (10 ng/µL)	0.1 µL	6 µL
Nuclease free water	add 1.00 µL	add 60.00 µL
Total volume	1.00 µL	60.00 µL

- Mix gently by vortexing and spin down shortly.
- Pipette 1 µL of the RT-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 RT-PCR on the AmpliSpeed slide cycler using your standard parameters. An example profile is given in table 2.

2 Table 2: Recommended thermal cycling conditions for RT-PCR amplification.

These guidelines are optimized for the Advantix AmpliSpeed slide cycler instrument. Annealing temperature should be optimized for each primer set based on the primer T_m . Skip the first two temperature steps when performing the One-Step reaction without DNase treatment. In case of skipping the DNase I digestion it is recommended to preheat the cycler to 42°C before inserting the AmpliGrid slide.

Temperature [°C]	Time
37°C	15 min Start RT-PCR + DNase I digestion
75°C	5 min
42°C	10 min Start One-Step RT-PCR
50°C	10 min
58°C	30 min
95°C	10 min
94°C	30 sec
55°C - 60°C	45 - 75 sec 35 - 45 cycles
72°C	45 - 75 sec
72°C	10 min
ambient	hold

OneStep RT-PCR with on-slide DNase I digestion

The protocol for RT-PCR with on-slide DNase I digestion is divided in two steps.

NOTE: Pre-Deposition of primers is not recommended for this protocol. Please add primers to the RT-PCR master mix as shown in table 4. Suggested final concentration of primers is 0.2 - 0.6 µM each. Optimization of primer concentration might be necessary especially for multiplex systems.

On-slide DNase I digestion setup:

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

3 Table 3: Composition of master mix for DNase I treatment

Component	Volume 1 rxn	Volume 48 rxn
DNase I Reaction Buffer	0.05 µL	3.0 µL
RNase inhibitor (10 U/µL)	0.01 µL	0.6 µL
DNase I	0.05 µL	3.0 µL
5x Single Cell RT Enhancer	0.15 µL	9.0 µL
Optional: total RNA control (10 ng/µL)	0.1 µL	6 µL
Nuclease free water	ad 0.50 µL	ad 30.00 µL
Total volume	0.5 µL	30.00 µL

RT-PCR reaction setup:

4 Table 4: Composition of master mix for RT-PCR after DNase I treatment

Component	Volume 1 rxn	Volume 48 rxn
2x Single Cell RT Reaction Buffer	0.60 µL	36.00 µL
Single Cell RT Enzyme Mix	0.05 µL	3.0 µL
Primer Mix (20 µM each)	0.01 - 0.04 µL	0.6 - 2.4 µL
Nuclease free water	add 0.7 µL	add 42 µL
Total volume	0.7 µL	42 µL

- 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 0.5 µL of the DNase I digestion 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.

- Start the programme shown in table 2 on the AmpliSpeed slide cycler.
- After reaching the 42°C step please add 0.7 µL of the RT-PCR master mix by pipetting either through or on top of the sealing solution. Please ensure not to create air bubbles during this pipetting step.
- NOTE: Do not remove the slide from the AmpliSpeed slide cycler during pipetting at the 42°C step.

Analysis & Storage

- For storage please transfer the AmpliGrid slide to an appropriate slide holder, e.g. the Advalytix slide tray and keep it at 4°C until further processing.
- For gel analysis please add 4 µL of a 1.5x concentrated gel loading buffer on top of the sealing solution.
- Aspirate the 5 µL sample volume by piercing the sealing solution with a pipette tip and transfer the samples to a gel (Agarose / PAA).

NOTE: After adding additional volume to the AmpliGrid please do not move the slide as the surface structure will not hold 5 µL volumes reliably.

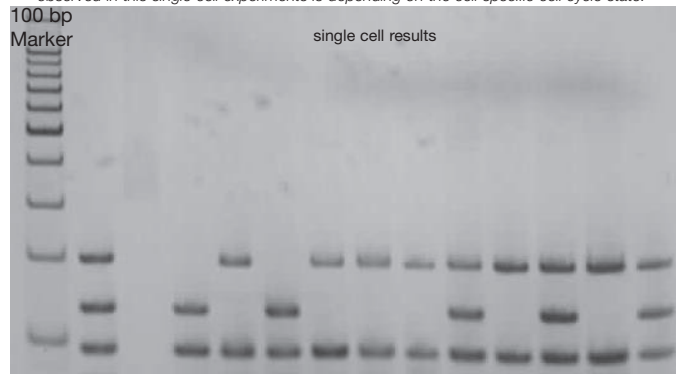
- For other downstream analysis methods please add 4 µL of ddH₂O instead of the gel loading buffer. It is also possible to retrieve the 1 µL sample but increasing the volume significantly reduces pipetting errors.

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The AmpliGrid system is intended for research applications and is not approved for clinical use!

1 Figure 1: Example results of a one-step RT-PCR. The lower lane shows a GAPDH housekeeping gene fragment amplified from human lymphocytes. The middle and upper lanes represent medium and low expressed genes. The heterogeneity for those genes observed in this single cell experiments is depending on the cell specific cell cycle state.



General Considerations

Single Cell RT Enhancer

The recommended concentrations of Single Cell RT Enhancer are a guideline and might have to be optimised depending on the individual biological system. Increase concentration in case of unspecific results. Decrease concentration in case of drop-outs. The recommended end concentration should be in the range of 0.1x to 1x Single Cell RT Enhancer.

RT-PCR temperature profile

The additional temperature steps of 42°C and 50°C at the beginning of the reaction help to generate more reliable results and higher yields by destroying secondary structures of template RNA.

Safety Precautions

Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for information regarding hazards and safe handling practice.

Xn: HARMFUL



Xi: IRRITANT

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