MANUFACTURED BY EURx

roboklon

dART
2-Step RT-PCR Kit
- for cDNA synthesis and RT-PCR -

dART cDNA Synthesis Kit
for Reverse Transcription (cDNA synthesis) and RT-PCR

Cat. No.	Size
E0802-01	25 reactions
E0802-02	100 reactions

Storage Conditions:

Store at -20°C

Unit Definition:

One unit is the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble form in 10 min at 37°C.

Two-Step RT-PCR: Protocol Overview:

First step (this kit): cDNA synthesis starts from either total RNA or from poly(A)$^+$-RNA. Primers are oligo(dT), random hexamers or reverse (anti-sense) gene specific primers.
Second step: Aliquots of the generated cDNA serve as template for PCR reactions in separate reaction tubes. Specific primer pairs are used for dsDNA amplification. Opti*Taq* DNA Polymerase, an enhanced proofreading *Taq*+*Pfu* enzyme blend, generates PCR products that can be used both for blunt or TA-cloning.

Quality Control:

All preparations are assayed for contaminating endonuclease and exonuclease and nonspecific RNase and single- and double-stranded DNase activities.

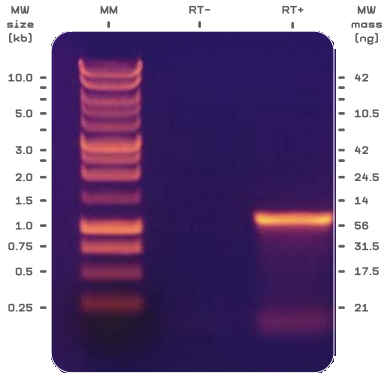

Fig. 1: 2-step RT-PCR for *Sus scrofa* type I arginase.
MW: PerfectPlus 1 kb Ladder [3.5 µl] (Cat. No. E3131),
RT-: Negative control, direct PCR from purified RNA without prior cDNA synthesis,
RT+: RT-PCR product of expected size (1.1 kb),
Detection limit: 1 ng RNA.

The dART kit is optimized for high sensitivity two-step RT reactions. It enables full length cDNA synthesis with high yield, even from rare, low copy number or delicate RNA templates. Featuring a carefully optimized, modified Reverse Transcriptase, the dART kit performs highly specific RT reactions.

Description:

- dART is a modified RNA dependent DNA polymerase, that synthesizes a complementary DNA strand from a single-stranded RNA in the presence of reverse (anti-sense) primers.
- For cDNA synthesis and for two-step RT-PCR reactions requiring high sensitivity and high specificity.
- Synthesizes single-stranded DNA from RNA templates in a broad range of temperatures between 35°C and 55°C.
- Greatly enhanced performance as compared to one-step RT-PCR reactions. No loss in RT sensitivity and no lowering of cDNA yield. Avoids any undesired compromises in reaction conditions, since RT and PCR reactions generally ask for very different buffer requirements.
- Ideal for amplifying whole genes for cloning and sequencing. High cDNA yield and full-length reverse transcripts for RNA templates with a wide range of G+C content. For sequence stretches with extremely high G+C content and very stable secondary structures we recommend using the thermostable AMV Reverse Transcriptase Native (Cat.No. E1372) at elevated reaction temperatures (55-65°C).
- No detectable RNase activity for single-stranded RNA. Reduced RNase H activity (DNA/RNA hybrid molecules only). RT reactions are non-amplifying reactions. Each RNA strand is reverse transcribed into cDNA exactly once, forming a RNA/DNA hybrid molecule upon completion. RNase H activity acts on these RNA/DNA hybrid molecules following reverse transcription and thus generally has no influence on cDNA product length and product yield.
- Suitable for preparation of labeled hybridization probes.

Package Contents:

Reverse Transcription / cDNA Synthesis

Reagents are provided for 25 or 100 cDNA synthesis reactions of 20 µl each.

Component	25 Rxn Kit	100 Rxn Kit
dART Reverse Transcriptase	25 µl	100 µl
5 x cDNA synthesis buffer	150 µl	600 µl
0.1 M DTT	50 µl	200 µl
dNTP mix [5 mM each]	150 µl	4 x 150 µl
RNase inhibitor (12.5 U/µl)	25 µl	100 µl
Oligo(dT)$_{20}$ (50 µM)	25 µl	100 µl
Random hexamers (50 ng/µl)	25 µl	100 µl
RNase free water	1.0 ml	4 x 1.0 ml
E.coli RNase H (2 U/µl)	25 µl	100 µl

PCR

Reagents are provided for 25 or 100 PCR reactions of 50 µl each.

Component	25 Rxn Kit	100 Rxn Kit
Opti*Taq* DNA Polymerase 2.5 U/ µl	25 µl	100 µl
10 x Pol Buffer C with $MgCl_2$	1.0 ml	4 x 1.0 ml
dNTP mix [5 mM each]	50 µl	200 µl

ROBOKLON GMBH | ROBERT-RÖSSLE-STR.10 B55 | D-13125 BERLIN | GERMANY
FAX 030 / 310 19 19 7 | PHONE 030 / 318 09 37 2 | WWW.ROBOKLON.DE
MANUFACTURED BY EURx Sp. z o.o. POLAND | WWW.EURX.COM.PL | MADE IN THE EU

MANUFACTURED BY EURx

roboklon

dART 2-Step RT-PCR Kit

TWO-STEP RT-PCR PROTOCOL

Preparation of Reactions:

(1) Primer / RNA /dNTP Mix

Component	Volume/Reaction	Final concentration
Primer Oligo $(dT)_{20}$ [50 µM] Rd. Hexamers [50 ng/µl] Reverse Prim. [10 µM]	1 µl	1x
RNA (10 ng – 5 µg)	x µl	0.5 - 250 ng/ µl
dNTP Mix [5 mM each]	4 µl	1 mM each
RNase free H_2O	To 13 µl	
Total Volume Primer / RNA /dNTP Mix	**13 µl**	

(2) Master Reaction Mix

Component	Volume/Reaction	Final concentration
5x cDNA Buffer	4 µl	1x
Ribonuclease Inhibitor, 12.5 U/µl	1 µl	0.625 U/µl
DTT [100 mM]	1 µl	5 mM
dART Reverse Transcriptase	1 µl	
Total Volume Master Reaction Mix	**7 µl**	

(3) PCR with Opti *Taq* DNA Polymerase

Component	Volume/reaction	Final concentration
Template DNA	2 - 5 µl	0.05 – 1 µg
10 x Pol Buffer C	5 µl	1x
dNTP mix (5 mM each)	2 µl	0.2 mM of each dNTP
Sense Primer [10 µM]	1 µl	0.5 µM
Reverse Primer [10 µM]	1 µl	0.5 µM
Opti *Taq* DNA Polymerase, 2.5 U/µl	1 µl	2.5 U
RNase free H_2O	To 50 µl	
Total volume	**50 µl**	-

(4) Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	93-95˚C	2-5 min	1
Denaturation Annealing Extension	93-95˚C 50-68˚C 72˚C or 68˚C	15-30 s 30 s 1 min/1 kb	20-40
Final Extension	72˚C or 68˚C	7 min	1
Cooling	4˚C	Indefinite	1

Reverse Transcriptase Reaction / cDNA Synthesis

This reaction allows synthesis of cDNA starting from small to medium RNA amounts. Depending on the abundance of the target RNA template in the RNA sample, from 10 ng up to 5 µg RNA permit the synthesis of full length cDNA.

1. **Prepare Primer/RNA/dNTP Mix:** Use a separate 0.2-0.5-ml plastic reaction tube for each reaction. In each tube, combine RNA, dNTP mix and primer (50 µM Oligo$(dT)_{20}$, or 50 ng/µl random hexamer primer or 10 µM reverse gene specific primer, respectively; composition see table 1). Adjust volume to 13 µl with RNase free water.
2. **Denature:** Heat Primer/RNA/dNTP mix 5 min at 65˚C and chill on ice for another 5 min.
3. **Mix Buffer:** Vortex the 5 x cDNA Buffer.
4. **Prepare Master Reaction Mix:** Prepare a master reaction mix on ice with 5x cDNA buffer, RNase Inhibitor, DTT and dART Reverse Transcriptase. Composition see table 2. Mix gently by pipeting on ice. The master reaction mix can be prepared in a single tube.
5. **Pool Both Mixes:** Transfer 7 µl aliquots of Master Reaction Mix into each of the ice-cooled tubes containing 13 µl Primer/RNA/dNTP mix (step 1).
6. **Incubate RT Reaction:** Transfer the sample to a thermal cycler preheated to an appropriate temperature. Incubate as follows:
   Oligo$(dT)_{20}$ primed : 30-60 min at 50˚C (or 35-55˚C)
   Gene specific primed: 30-60 min at 50˚C (or 35-55˚C)
   Random hexamer primed: 25˚C for 10 min, followed by 20-50 min at 50˚C (or 35-55˚C).
7. **Terminate RT Reaction:** Terminate the reaction by heat inactivation at 85˚C for 5 min.
8. **RNase H Digestion:** Add 1 µl of RNase H and incubate at 37˚C for 20 min (optional). Note: RNase H activity digests DNA/RNA hybrid molecules only.
9. **Finish cDNA synthesis:** cDNA is ready for PCR, can be used immediately or stored at -20˚C

PCR Reaction with Opti *Taq* DNA Polymerase

1. **$MgCl_2$:** Use 2-5 µl of the cDNA as a template for PCR. The final magnesium concentration is 1.5 mM in reaction. In some cases there is a need of titration of magnesium to obtain best results. Adding 1 µl of a 25 mM $MgCl_2$ solution to a total reaction volume of 50 µl will add 25 nmol $MgCl_2$ and thus increase total $MgCl_2$ reaction concentration in 0.5 mM.
2. **Mix:** Mix all components for PCR amplification in a 0.2 – 0.5 µl plastic reaction tube (Composition see table 3). Mix all components gently by pipetting.
3. **PCR:** Incubate at 94˚C for 3 min, then perform 20-40 cycles of PCR with optimized conditions for the samples (1 min/kb extension time at 68-72˚C). See table 4.
4. **Gel Electrophoresis:** Analyze 10-20 µl of PCR sample by agarose gel electrophoresis.

Logsheets

Standardized log sheets for RT- and for PCR- reactions are available online:

RT
http://www.roboklon.com/rt
PCR
http://www.roboklon.com/pcr