EURx® MOLECULAR BIOLOGY PRODUCTS

Positive Control Set for RT-PCR

Cat. No.	size
E0820-01	20 reactions

Positive Control Set for RT-PCR provides RNA template and primers dedicated for positive verification of the first strand cDNA synthesis step for smART series (E1376, E0804, E0805). The human GAPDH control synthetic RNA (1.2 kb) was produced by *in vitro* transcription and contains poli A tail at 3'end. The GAPDH-specific control PCR primers are designed for amplification of 496 bp RT-PCR product.

Storage Conditions: Store at -20°C.

Quality Control:

All preparations are assayed for contaminating endonucleases, exonucleases, nonspecific RNases, single- and double-stranded DNase activities.

COMPONENT:	E0820-01
Control GAPDH RNA 1 µg	lyophilized
Forward GAPDH Primer 10 µM	20 µl
Reverse GAPDH Primer 10 µM	20 µl

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I Control first strand cDNA synthesis reaction*

Mix and brifely centrifuge all components after thawing, suspend Control GAPDH RNA in 20 µl (50 ng/µl) of nuclease free water**, keep on ice.

1. Assambly reaction in RNase-free tube as follows:

Component:	Amount:
Control GADPH RNA (50 ng/µl)	1 µl
Oligo $(dT)_{18-20}$ Primer 50µM or Random Hexamer Primer 200 ng/µl or Reverse GAPDH Primer 10 µM	1 µl
dNTPs Mix 10 mM each	1 µl
5 x cDNA Buffer	4 µl
DTT 100 mM	2 µl
Ribonuclease Inhibitor 50 U/µl E4210	0.5 µl
smART (200 U/µl)	1 µl
RNase-free Water	to 20 µl
Total volume	20 µl

2. Mix gently and centrifuge.
3. Transfer the sample to preheated to appropriate temperature thermal cycler.
   Incubate as follows:
   Oligo$(dT)_{18-20}$ primed: 20-60 min at 50°C (or 37-65°C)
   Reverse GAPDH primed: 20-60 min at 50°C (or 37-65°C)
   Random Hexamer primed: 25°C for 10 min, followed by 20-50 min at 50°C (or 37-65°C).

4. Terminate the reaction by incubating at 85°C for 3 min.
5. cDNA is ready for PCR amplification, can be used immediately or stored at -20°C.

Notes:

* negative controls w/o reverse transcriptase and w/o template for exluding genomic DNA contamination and confirming reagents purity should be also performed.

**be careful when opening tube with the fluffy RNA pellet to not loose it. Make sure that RNA pellet is nicely dissolved and centifuge brifely. Once the RNA is resuspended in nuclease-free buffer, it should be stable at -20°C (in the dark if fluorescently labeled) for at least six months assuming it is kept RNase-free. It is best to store resuspended RNA in aliquots to reduce the effects of any accidental RNase contamination.

II PCR amplification with OptiTaq DNA Polymerase

1. Dilute the cDNA generated with the control first strand cDNA reaction 1:100 in nuclease-free water.
2. Mix gently and brifely centrifuge all PCR reagents after thawing.
3. Assambly reactions on ice in nuclease-free 200 µl PCR tubes as follows:

Component:	Amount:
cDNA from control RT reaction (1:100 dilution)	2 µl
10 x Pol Buffer C	5 µl
dNTPs Mix 10 mM each	1 µl
Forward GAPDH Primer 10 µM	1 µl
Reverse GAPDH Primer 10 µM	1 µl
OptiTaq DNA Polymerase 5 U/µl E2600	0.5 µl
RNase-free Water	39.5 µl
Total volume	50 µl

4. Perform PCR amplification in thermal cycler according to program:

Step	Temperature	Time	Number of Cycles
Pre-denaturation	94°C	3 min	1
Denaturation	94°C	30 s	35 cycles
Annealing	55°C	30 s	
Extension	72°C	30 s	
Final Extension	72°C	2 min	
Cooling	4°C	Indefinite	1

5. Load 10-20 µl of the RT-PCR product on 1.5% agorose gel. A distinct 496 bp PCR product should be visible after ethidium bromide staining.

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